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ELECTROPHORETIC SEPARATOR FOR PURIFYING BIOLOGICALS

FINAL REPORT PART I

Prepared for:
GEORGE C. MARSHALL SPACE FLIGHT CENTER

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GENERAL ELECTRIC COMPANY
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SPACE SCIENCES LABORATORY

ELECTROPHORETIC SEPARATOR FOR
PURIFYING BIOLOGICALS

FINAL REPORT

PART I

CONTRACT NAS 8-31036

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FOREWORD

The Electrophoretic Separator for Purifying Biologicals program was originated under the Advanced Applications Flight Experiment (AAFE) series of engineering model level developments at NASA-Langley Research Center in response to an Announcement of Opportunity in the spring of 1973. The responsibility for contracting and technical management was transferred to the Marshall Space Flight Center as part of their Materials Processing in Space program, under which Contract NAS 8-31036 was then initiated on August 13, 1974 with the General Electric Company.

Many people in NASA, the pharmaceutical industry, the academic community, and General Electric have participated and aided in the development of space processing of biologicals by electrophoresis and particularly of this unique apparatus and related science and technology. While there is a risk in citing some and omitting others, it is appropriate to mention the following as having a particularly high degree of involvement and offer the author's thanks and apologies to those who are not specifically named:

NASA: Dr. R. E. Allen - Dr. James H. Bredt - Lloyd Gardner -
E. C. McKannan - Robert L. Parker - P. H. Rhodes -
Dr. R. L. Snyder

GE: F. Cosmi - Dr. J. A. Giannovario - E. L. Gray -
Dr. R. N. Griffin - R. Grosso - R. J. Locker -
E. Muziani - H. W. Semon - M. Wagner

ABSTRACT

A four year program to develop an engineering model of an electrophoretic separator for purifying biologicals has been successfully completed and is summarized in this report.

The program included an extensive mathematical modeling study and numerous ground based tests. It was principally focused however on developing an actual electrophoretic separator of the continuous flow type, configured and suitable for flight testing as a Space Processing Applications Rocket payload.

Extensive reporting, and publication of the various aspects of the program has been accomplished along with the preparation of drawings, sketches and an Operating Manual which have all been furnished with the actual experiment hardware to the Marshall Space Flight Center. These are briefly reviewed and referenced to serve as a guide to those interested in the various aspects of the program.

During the last year of the program a series of special tests and studies related to potential flight experiments was conducted under a separate task and will be reported separately.

I. INTRODUCTION

The idea for performing electrophoretic separation of biologicals in space came from Wyeth Laboratory scientists at a meeting of General Electric Space Sciences Laboratory scientists who were visiting them in the spring of 1969. It was based on a simple and elegant statement that the best and most widely used analytical technique for biologicals cannot be scaled to prepare larger more useful quantities of biologicals due to gravity imposed limitations. A small or thin cell can be operated at low power by cooling and orienting it properly on earth, but the throughput is unacceptable for practical applications.

Subsequent discussions both at Wyeth and at numerous other organizations, such as NIH, indicated there were several important biologicals such as certain vaccines, erythropoietin, and AHF which users would benefit from having in purer form.

Over the following nine years numerous consultations, studies, conferences, and a few flight demonstrations and tests have been conducted but primarily on other ideas associated with separating cells which are of interest for various research projects.

Meanwhile ideas for equipment and techniques for performing separations also proliferated. They were generally not specific to either the separation of cells or proteins and could therefore be considered general purpose. However, they usually required considerable basic development in addition to the necessary adaptations required to make them automated or otherwise adapted to space operation.

The approach adopted in our work has instead been to select the previously developed, relatively simpler techniques of continuous free flow electrophoresis, which are clearly limited by gravitationally induced convection, and adapt them to space operation. This has been done both by Hannig in the MA-014 experiment on ASTP and in the work to be briefly reviewed in this report.

Before describing this program, the nature of the problem should be further illustrated. Electrophoresis is a very widely practiced art in the analysis of biologicals (as well as in other fields such as for preparing coatings). It is usually performed and used by experts in the biological sciences in a rather expeditious manner - which implies fresh reagents and great care to maintain sterility and viability as needed. These conditions are obviously very difficult to attain or maintain in the space flight regimes where schedules alone have usually required the preparation and storage (sometimes unrefrigerated) for weeks to months. Not only is the effect of delicate solutions on the surface chemistry of samples and membranes detrimental but is unknown. Much of the effort therefore has been aimed at arriving at suitable techniques, materials, and operational procedures for performing electrophoresis in space. It is not yet complete and needs specific flight tests to ascertain the progress and to help in defining the potential benefits of space processing biologicals.

In this report the overall development of an engineering model of a free flow electrophoresis separator experiment (ESE) along with both analytical

and experimental work plus numerous tests and supporting activities is briefly described. Since the actual engineering test hardware, supporting documents and drawings plus several technical papers have been supplied, it is considered redundant to describe or reproduce all of these items in a single large report. Instead they are briefly described and referenced, so this report will serve as a roadmap of this four year program.

The report starts with the six statements of objectives for the work and then indicates the progress toward fulfilling each of them.

II. OBJECTIVES OF THE PROJECT (REPRODUCED FROM THE CONTRACT SCOPE OF WORK)

Title: Electrophoretic Separator for Purifying Biologicals

I. Objective: The basic objectives of the project are:

1. Develop the analytical and experimental basis for a thick cell, free-flow electrophoretic separator for application in the low gravity of space.
2. Design, build, and test an engineering model of the electrophoretic separator.
3. Demonstrate the experiment operation on the ground with simulated weightless conditions such as density gradients, model particles, etc.
4. Operate the model and conduct experimental simulation studies to provide data and experience that will specify the performance of the design when translated to the space environment.
5. Predict the operation in space based upon analysis and experimentation. Identify problem areas that remain unresolved by ground evaluation that must be tested or measured in space.
6. Define flight experiments of varying duration from several minutes to several hours that will demonstrate and prove the performance of the design and apparatus concepts.

III. DESCRIPTION OF THE WORK, RESULTS AND STATUS

1. Analytical and Experimental Basis for a Thick Cell

This was a major portion of the program which resulted in an excellent computerized math model of the continuous flow, thick (5mm) electrophoresis cell. A technical paper on this portion of the work is being published⁽¹⁾.

A second major portion of this work was performed on a General Electric Company purchased commercially available continuous flow electrophoretic separator which was extensively modified to, for example, permit using biologicals in it. Several different biologicals were separated in it and compared with the mathematical model. This was partially in cooperation with several commercial and governmental laboratories who supplied samples, consultation, and further analysis. An important part of this supplementary effort was supplied by grants from the National Science Foundation, Faculty Research Participation Program which supported three professors for ten weeks each in the summers of 1975 and 1976. They studied various aspects of the separation or purification of the following materials in conjunction with the indicated organizations:

| <u>Material</u> | <u>Professors</u> | <u>Cooperating Laboratory</u> |
|-------------------------------|--|--|
| Influenza B Virus Vaccine | Dr. Lyman Magee Ms. Marian Johnson | Wyeth Laboratories |
| Erythropoietin | Dr. Vito DelVecchio | NIH & Children's Hospital-Los Angeles |
| Kidney Cells- Urokinase | Dr. Edward Peeples | Abbott Laboratories |
| Swine Flu Sub-Unit Vaccine | Dr. Gaylord E. Shaw | Wyeth Laboratories |
| Animal Sperm | Dr. Arthur Wu | U. S. Dept. of Agriculture Wyeth Laboratories |
| Plant Cells | Dr. E. John Staba (GE & NASA sponsored) | University of Minnesota |

Portions of this work are still to be published while other portions have already been reported in several quite different forums (2,3,4).

2. Engineering Model of the Electrophoretic Separator

This was extremely well done in two different models and in a configuration for flight testing on the Black Brant Sounding Rocket or later use on the Space Shuttle. The two different models are shown in Figures 1a and 1b and are distinguished by the original model (Figure 1a) being equipped with a camera for observing the separated sample streams through a cross section analyzer which is useful for studying the operation of the equipment when using an opaque model material such as polystyrene latex. The later model (Figure 1b) incorporates a UV sensor and a passively refrigerated sample collection system for maintaining up to 50 separated fractions of either model or biological materials.

Other features common to both models include:

- a) Passive refrigeration for cooling the electrophoresis chamber, buffer, and electrolyte for about six hours.
- b) Widely adjustable pump speeds, field voltages, and other experimental conditions to permit the performance of separations at many different conditions.
- c) Telemetry provisions for both experiment and equipment performance data, e.g. UV sensor, temperature, pump voltages which are calibrated to flow rates, etc.
- d) Dry atmosphere to prevent moisture condensation on windows.
- e) Long term operation on earth or shuttle flights by providing power, gas, chilling fluids, and additional buffer through external connectors (shown on the bulkhead in the bottom of the pan, Figures 1a and 1b).
- f) Complete control and operating panel with extra long cables for use in sounding rocket assembly and launch operations.

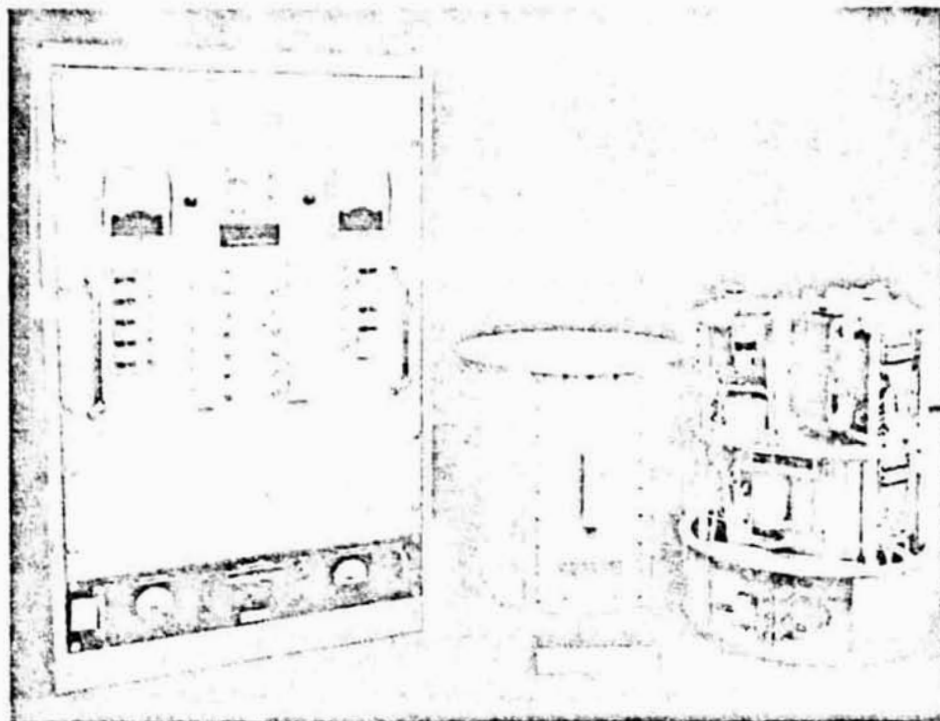


Figure 1a. Original Configuration of Electrophoretic Separator Experiment (ESE) with camera for data acquisition on right.

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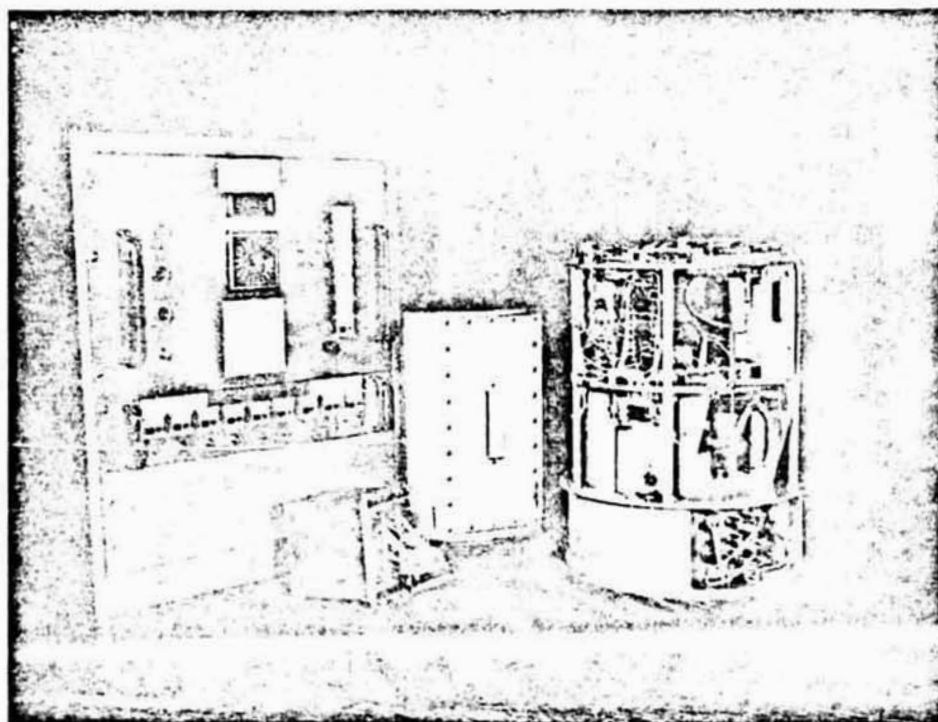


Figure 1b. Current Configuration of ESE shown with sample collection box in middle foreground from upper middle front of unit. Note separate control and operating panels, connecting cables and access door in shroud.

3. Ground Based Operation

Numerous engineering tests to develop and assess the operation of components as well as the entire system were performed. These culminated in the successful functional and vibration testing to simulate a Nike boosted black Brant Sounding Rocket in May 1978.

Of technical interest to the overall program however are various tests performed most recently in the spring of 1977 and reported in the June 1977 monthly report. These tests assessed the power level in watts versus residence time in seconds during which the unit could be operated in various orientations before the flow became unacceptably disturbed by convection. Figure 2 is from that report and shows the results which are admittedly somewhat subjective in terms of the judgment of how long the equipment can operate at each setting before the sample stream has meandered too much. However, of greater importance is that the power input to cause these unacceptable instabilities of the sample stream are one to two orders of magnitude lower than the power settings to be used in low g where the convection should be minimized.

An illustrated operating manual⁽⁵⁾, plus about 80 drawings and sketches have also been prepared and supplied to NASA-Marshall Space Flight Center.

4. Operate the Model and Conduct Experimental Simulation Studies

Between the many hours of engineering development tests, followed by environmental (shock and vibration) tests and the experiment simulation tests (previously described above) the ESE has had several hundred hours of operation. They have both confirmed the design and indicated the useful life of some components and materials. This had led to various suggestions e.g. the need for a space peristaltic pump, replumbing, more reliable electrode membranes, etc. for increasing the reliability of the unit.

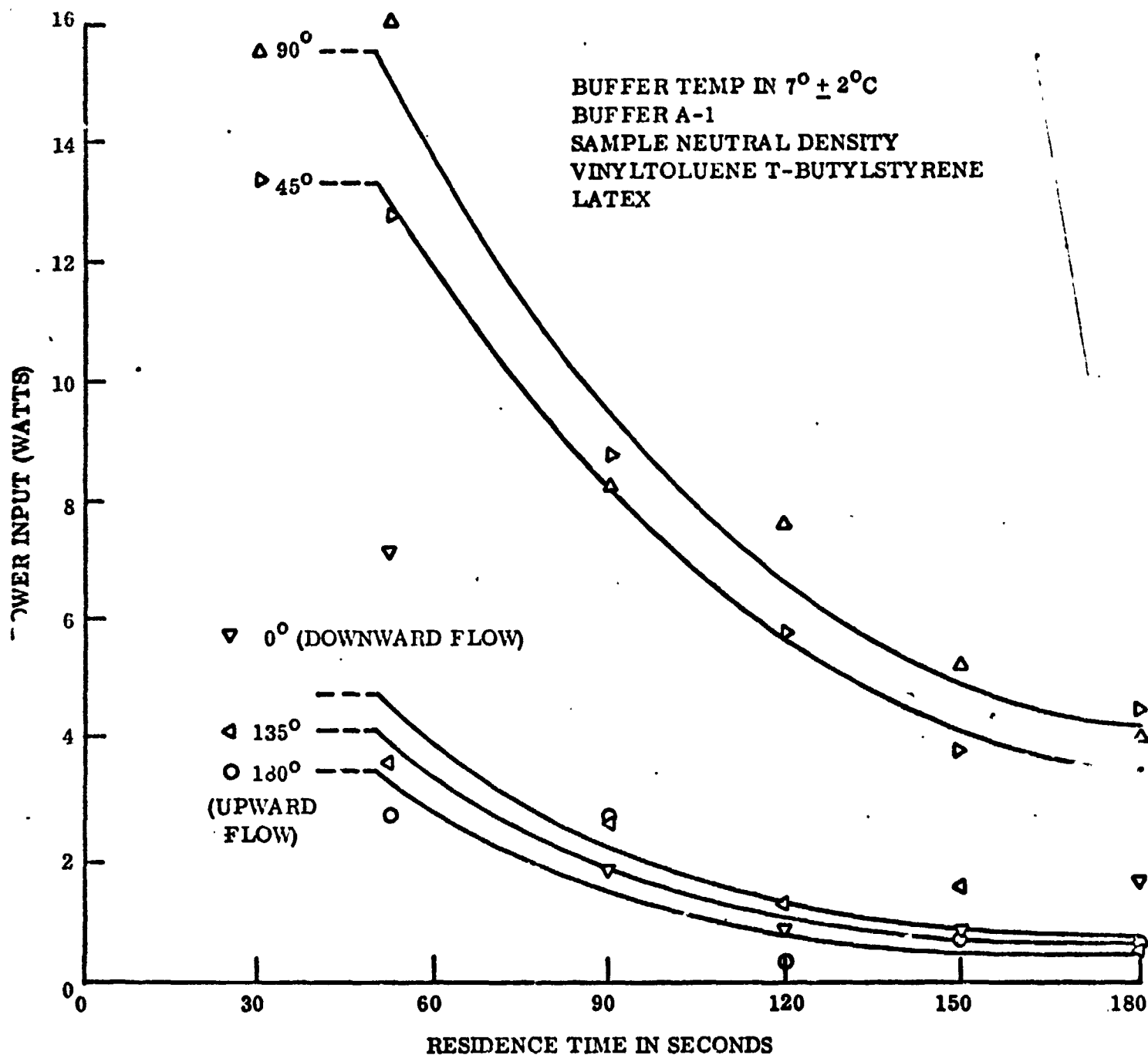


Figure 2. Effects of Orientation and Residence Time SPAR Electrophoresis Chamber
 on Maximum Usable Power Under Cooled Conditions
 (Power Settings above curves lead to instability of the sample stream.)

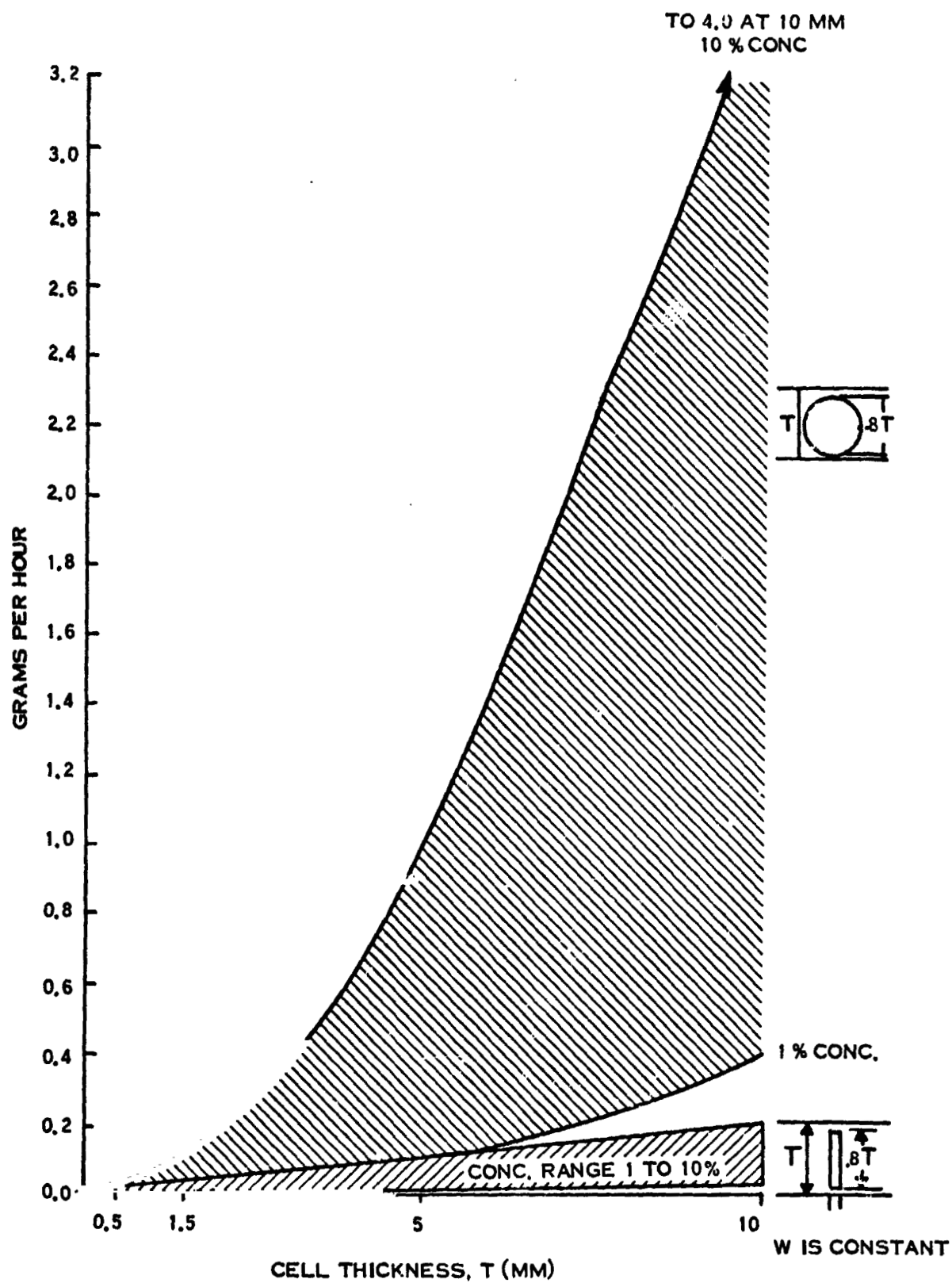


Figure 3 Calculated throughput for continuous electrophoretic separators as a function of cell thickness and sample concentration for high resolution (lower curves) and high throughput examples.

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A MATHEMATICAL MODEL OF FREE-FLOW ELECTROPHORESIS

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SUMMARY

A computerized mathematical model has been developed of a free-flow electrophoresis cell operating under conditions of no convection and no sedimentation of sample. The complex interactions of the various system parameters have been identified and included in this model. Data inputs representing existing equipment have been processed with the theoretical results comparing well with experimental results. Data were also processed for an experimental electrophoresis cell designed to allow optimum resolution and/or sample throughput while operating in a zero g environment. Theoretical results are presented along with some experimental ground-based data.

INTRODUCTION

The fact that particles dispersed in a solution could be influenced by an electric field was first described by Ledge¹ in 1886. Six years later, Picton and Linder² related their systematic studies of the phenomenon. However, as with most new techniques, there was a dormant period, and it was not until the work of Tiselius³ in 1937 that electrophoresis began to receive increased attention. The Tiselius method was originally of interest only to biochemists and medical researchers. However, with the introduction of lower cost equipment and advances such as supporting media, biologists, chemists and engineers use the technique for analysis, separation, identification and purification.

During the nineteen fifties and sixties, men such as Barrolier *et al.*⁴ and Hannig⁵ proposed preparative electrophoretic techniques based on a flowing system in which both the buffer and the sample were continuously admitted to the electrophoresis chamber, with the separated fractions being collected in individual containers. Such electrophoresis systems are now categorized as "free-flow". Because these systems are made thin to minimize convection problems and to maintain stable temperature gradients, the sample fractions are generally distorted due to both hydro-

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dynamic and electro-osmotic flow profiles. These types of distortion were recognized by Kolin⁶ in his magnetically driven electrophoretic separator, and by Strickler and Sacks⁷ and Hannig *et al.*⁸ in the usual free-flow electrophoresis systems.

Distortion of the sample bands can be decreased by decreasing the thickness of the sample stream or by increasing the thickness of the electrophoresis cell. In principle the sample stream thickness could approach zero; in practice the thickness is a significant fraction of the cell thickness. An alternative method of obtaining less distorted sample bands is to increase the electrophoresis cell thickness. This would result in "flatter" profiles for both buffer curtain flow and electro-osmotic flow, but would aggravate the problem of convection since the temperature difference would be greater in a thicker cell. A thin cell can be expected to have severe distortion of the sample due to steep flow profiles, while a thick cell will suffer from convection problems.

A solution is possible. Since convection and sedimentation are attributable to a gravity field, these problems may be alleviated, at least theoretically, if the cell system were to be operated in a zero *g* environment. The advantages would be: a thicker cell to flatten the flow profiles and the absence of convective mixing and sedimentation of samples at high concentration.

This work describes a mathematical model of an electrophoresis cell which operates under the conditions of no convection and no sedimentation (absence of gravitational effects).

THEORETICAL FOUNDATION

General

In electrophoresis the item of interest is the rate of migration. Provided the migration path is of sufficient length a mixture of components may be separated. The rate of migration (electrophoresis) is a function of net charge, size and shape of the particles, and retarding factors such as viscosity. A particle which has no net charge or is uncharged should not migrate. However, a liquid flow occurs, induced by the applied field, which causes all species present to migrate. This is known as electro-osmosis.

The following sections will discuss the ζ -potential, electrophoretic velocity and mobility, and electro-osmosis.

ζ -Potential

The charge and potential near a phase boundary have been considered in detail by Debye and Hückel⁹, Audubert¹⁰, Gouy¹¹, Chapman¹², Stern¹³ and others. The application of these equilibrium properties to electrokinetics has led to the concept of a "slipping plane" displaced somewhat from the actual phase boundary. Electrokinetic phenomena are controlled by the potential at this slipping plane called the ζ -potential as indicated in Fig. 1. The concept of the slipping plane and its attendant potential is useful in measurements and calculations relating to electrophoresis, but the relationship to more fundamental properties of the phase boundary is somewhat tenuous. More detailed descriptions of ζ -potentials and their application in electrophoresis can be found in refs. 14-17, and in the many references cited therein.

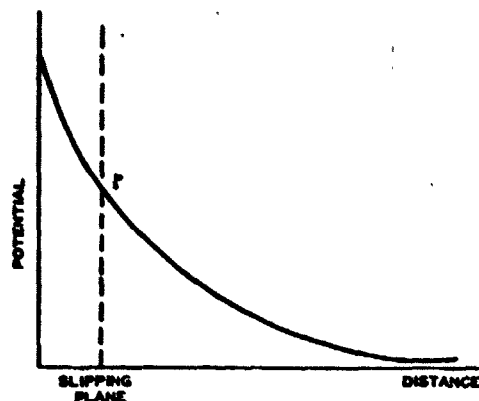


Fig. 1. Variation of potential with distance from a charged surface.

Electrophoresis and mobility

Elementary analysis indicates that if an electric field, E , is applied to a particle of net charge C , the force producing electrophoretic migration is EC . The resisting force is given by Stokes' law, i.e., $F = 4\pi\eta aV$ for a spherical particle where a is the particle radius, η is the bulk viscosity of the medium and V is the particle velocity. If the particle has mass m , and neglecting electrostatic interactions, the motion is described by

$$EC = \frac{md^2x}{dt^2} + 4\pi\eta a \frac{dx}{dt} \quad (1)$$

The transient response is rather small (10^{-14} sec) and the particle accelerates to its limiting velocity almost instantly. The limiting velocity or the electrophoretic velocity (V_{ep}) is given by:

$$V_{ep} = \frac{EC}{4\pi\eta a} \quad (2)$$

The mobility μ (velocity in unit field) is given by:

$$\mu = \frac{V_{ep}}{E} = \frac{C}{4\pi\eta a} \quad (3)$$

and it can be shown that the mobility is related to the ζ -potential in the following manner:

$$\mu = \frac{\zeta D}{4\pi\eta} \quad (4)$$

where D is the dielectric constant of the solution.

It has, of course, been shown that the constant, 4π , in eqns. 1-4 is valid only when the radius of the phase boundary is large compared to the thickness of the

electrical double layer. Under other circumstances the constant can range up to $6\pi^{2,19}$, depending on particle size and the composition and ionic strength of the surrounding medium. For our present purpose the use of the constant, 4π , will suffice. The extension to other circumstances is obvious.

Electro-osmosis

The phenomenon known as electro-osmosis is due to the potential difference existing between the wall of a chamber and the layer of liquid lying next to it; that is to the double layer at the boundary between solid and liquid. The application of an electric field must cause a displacement of the charged layers, and since the wall cannot move the liquid must, and a flow results. The direction of flow depends on whether the ions in this double layer are positive or negative.

Suppose that the wall of the chamber is negatively charged and the layer of liquid adjacent is positively charged. If a field E is applied and the surface density of charge is given by σ the force acting on unit surface is $E\sigma$. The viscous forces opposing flow are given by $\eta(V_{eo}/\delta)$ where δ is the double layer thickness and V_{eo} is the electro-osmotic velocity. For a steady flow the two forces must be equal.

$$E\sigma = \eta \frac{V_{eo}}{\delta} \quad (5)$$

As in electrophoresis, the electro-osmotic velocity at the wall, V_{eo} , can be related to ζ -potential (of the wall) and is given as

$$V_{eo} = \frac{\zeta_w DE}{4\pi\eta} \quad (6)$$

where D is the dielectric constant of the solution, η is its viscosity and ζ_w is the ζ -potential of the wall surface with respect to the bulk solution. The fundamentals of electro-osmosis in a closed system are well known¹⁹, and while a free-flow electrophoresis system is, by definition, not a closed system it is closed in the direction of electro-osmosis and the recirculation characteristic of a closed system is observed. Nee has recently re-examined in detail the fundamental equations describing electro-osmosis²⁰.

ASSEMBLY OF THE COMPUTERIZED MODEL

Introduction

A useful electrophoresis system designed to operate in a zero-g environment should be flexible enough to handle some of the very different biological materials which remain unseparated by present terrestrial electrophoretic methods. The resolution necessary to obtain useful material will vary for each species. This implies an electrophoresis unit with considerable operational latitude in sample flow-rate, sample residence time, field potential, wall ζ -potentials and separation resolution.

The entire mathematical model is based on a criterion called the separation resolution and defined as $\Delta\mu$, the minimum difference in sample component mobility which will result in the complete separation of two adjacent sample components by

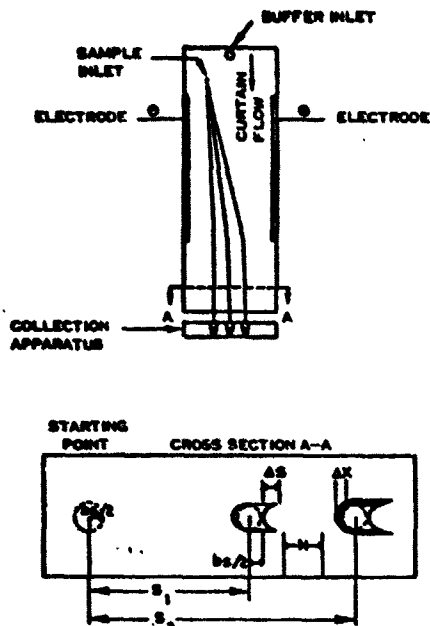


Fig. 2. Schematic, free-flow electrophoresis and definition of separation resolution.

an amount equal to the spacing of the product fraction collection tubes. This is illustrated in Fig. 2.

The term ΔS is the crux of the matter. This term is calculated by taking a sample particle at two locations on the outer edge of the sample stream and calculating net displacements at those points. Fig. 3 illustrates the concept.

The displacement at either point is given by

$$S_x = V_{net(A)} \cdot t_{r(A)} = (V_{ep(A)} + V_{eo(A)}) \cdot t_{r(A)} \quad (7)$$

$$S_0 = V_{net(B)} \cdot t_{r(B)} = (V_{ep(B)} + V_{eo(B)}) \cdot t_{r(B)} \quad (8)$$

$$t_r = L/V_b \quad (9)$$

$$\Delta S = S_x - S_0 = [(V_{ep(A)} + V_{eo(A)}) t_{r(A)} - (V_{ep(B)} + V_{eo(B)}) t_{r(B)}] \quad (10)$$

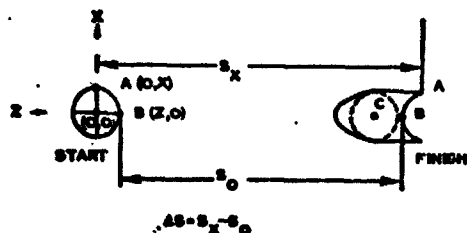


Fig. 3. Definition of sample distortion, ΔS .

where

- V_{ep} = electrophoretic velocity,
 V_{eo} = electro-osmotic velocity,
 V_{net} = algebraic sum of electrophoretic and electro-osmotic velocities,
 t_r = residence time in the field,
 V_b = the buffer velocity at x and
 L = length of the field.

Eqn. 10 is simplified in that it does not take into account diffusion effects. If diffusion were to occur with displacements on the order of the electrophoretic displacements then the A term would be constantly changing during the time particle A is in the field. This effect is built into this model.

In eqn. 10, all of the velocity terms have a dependence upon viscosity, and viscosity is in turn dependent upon temperature. Thus, temperature becomes the most important parameter in the model for separation resolution. Temperature is also important to sensitive (biological) materials, and it is probably necessary to keep the maximum temperature at or below physiological temperature (37°). Therefore, before an attempt can be made to calculate any of the velocities (V_{ep} , V_{eo} , or V_{net}) it is necessary to determine the temperature profile through the cell thickness and the maximum temperature at the cell centerline.

It is now possible to take an overview of the system and identify the interdependence of the cell variables. Fig. 4 shows this interdependence.

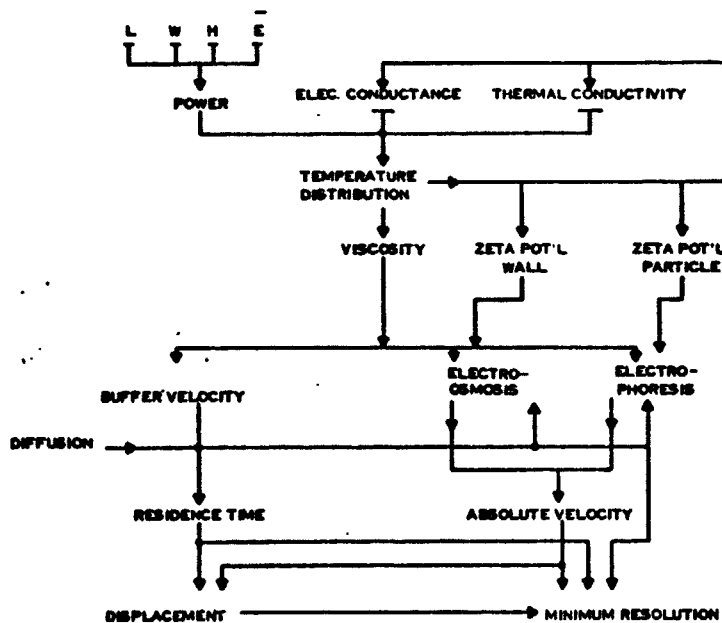


Fig. 4. Interdependence of cell variables.

Temperature distribution and cell thickness: step 1

As mentioned before, it is the cell thickness that is the crucial factor in a successful free-flow system. This is due to several factors: (1) heat transfer occurs through this dimension; (2) the hydrodynamic flow profile is determined by the cell thickness; and (3) the extent of electro-osmotic distortion is determined by the thickness also. The most important parameters here are the temperature distribution and the maximum temperature at the cell centerline. Since these increase rapidly with cell thickness, a trade-off must be made between large temperature gradients and flatter flow profiles.

The first step in the development of the model was to describe accurately the temperature gradient through the cell thickness and from the maximum temperature at centerline to choose an appropriate cell thickness. A similar analysis was performed by Brown and Hinckley²¹ subsequent to completion of this work. Their conclusions were generally similar to ours except that we did not consider the wall thickness. In designing equipment we strive for the highest practical thermal conductivity in the walls. Some standard textbook equations were solved first to acquire a "feel" for the solutions (see Figs. 5A, 5B and 6A). Fig. 6B shows the data resulting from the finalized mathematical description of the temperature distribution.

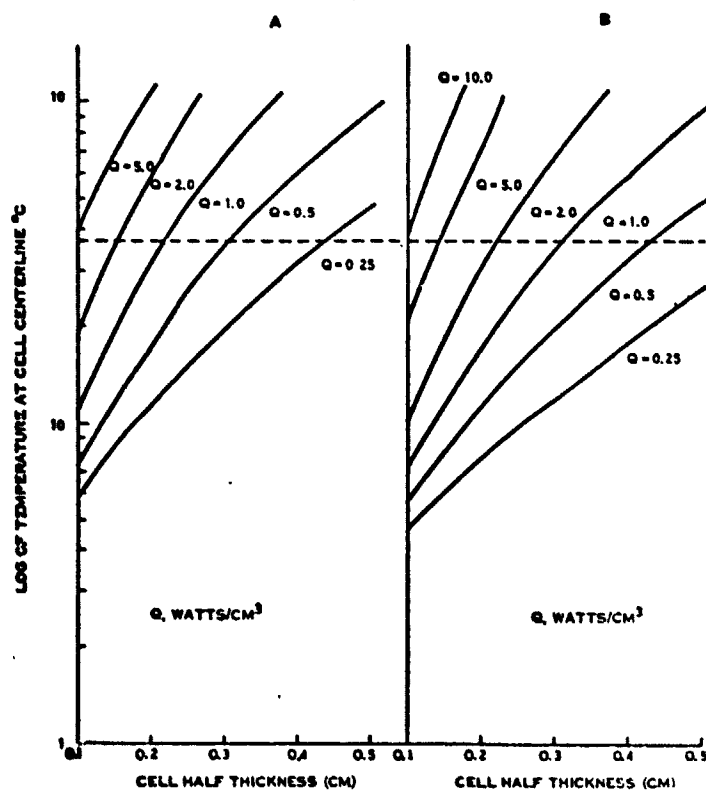


Fig. 5. A, planar heat source; B, distributed heat source, both cases having fixed thermal and electrical conductivities.

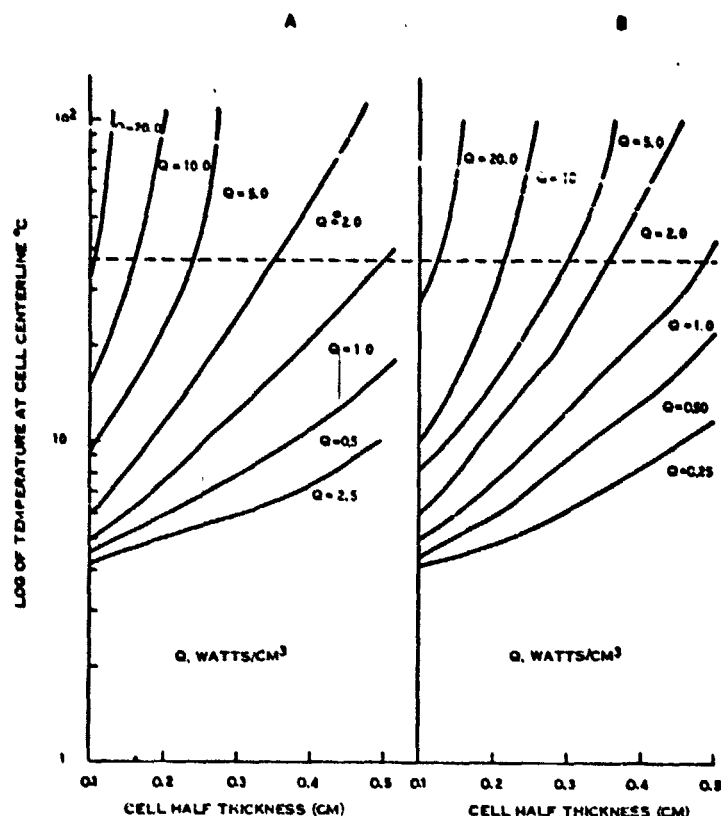


Fig. 6. A, distributed heat source with fixed thermal conductivity and variable electrical conductivity. B, Distributed heat source having variable thermal and electrical conductivity.

The equation governing this distribution may be written as

$$(a + \beta y) \frac{d^2 y}{dx^2} + \beta \left(\frac{dy}{dx} \right)^2 + Q(1 + \epsilon y) = 0 \quad (11)$$

where y = temperature difference from wall at x , x = distance from cell centerline, a = thermal conductivity of buffer at 4° , β = temperature coefficient of the buffer thermal conductivity, ϵ = temperature coefficient of the buffer electrical conductivity and Q = power density in W/cm^3 containing both the field, E , and the buffer electrical conductivity, ke .

Eqn. 11 is a boundary value problem (of the second kind) rather than an initial value problem. The boundary conditions for eqn. 11 are: the derivative of the temperature at the cell centerline be equal to zero, $y'(0) = 0$ and the temperature difference at the wall (x_f) be equal to zero, $y(x_f) = 0$. The distribution is assumed to be a symmetrical function with respect to the cell centerline. The sought-for value is the temperature at the cell centerline, $y(0)$. Some sort of iterative technique must be used to solve this equation, with the additional condition that the solution converge

reasonably rapidly. The method used here is a variant of the so called "shooting-method", in which a value for $y(0)$ is assumed, and with $y'(0) = 0$, the equation is then integrated over $[0, x_f]$, and a $y(x_f)$ is calculated. This result, $y(x_f)$, is compared with the condition $y(x_f) = 0$, and the comparison is used to derive a better estimate of $y(0)$. The process is repeated until successive iterations converge. It is clear that some mechanism must be provided to establish how much $y(0)$ is to be incremented on a given iteration and the Newton-Raphson technique was chosen for this purpose.

Referring to Figs. 5 and 6, note how each refinement of the model has affected the maximum temperatures at the cell centerline. From these data a cell thickness of 0.5 cm (0.25 cm half thickness) was chosen. The horizontal dashed line on each graph represents physiological temperature, 37° . An additional output from this step is the temperature profile through a cell of a given thickness. Figs. 7, 8 and 9 are examples of this output for cells of thickness 0.07 cm, 0.16 cm and 0.5 cm, respectively. These data are read onto a file and used in subsequent calculations.

The data inputs to step one are: buffer conductivity and its temperature coefficient, thermal conductivity and its temperature coefficient, voltage gradient and one-half the cell thickness.

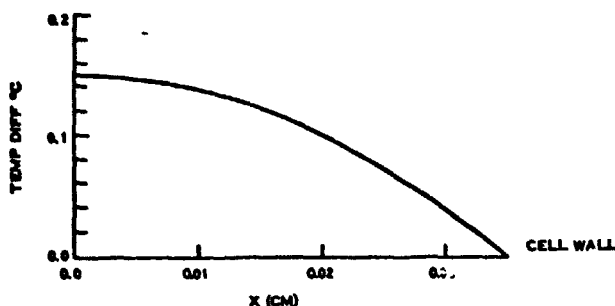


Fig. 7. Temperature gradient vs. distance from cell centerline for a field of 40 V/cm and a conductance of $8.7 \times 10^{-4} \Omega^{-1} \text{cm}^{-1}$. Cell thickness, 0.07 cm.

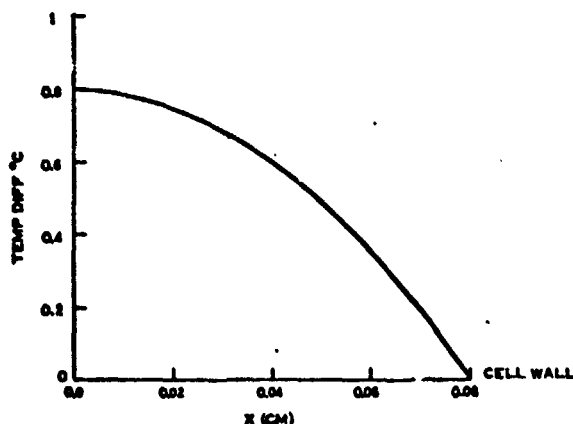


Fig. 8. Temperature gradient vs. distance from cell centerline for a field of 40 V/cm and a conductance of $8.7 \times 10^{-4} \Omega^{-1} \text{cm}^{-1}$. Cell thickness, 0.16 cm.

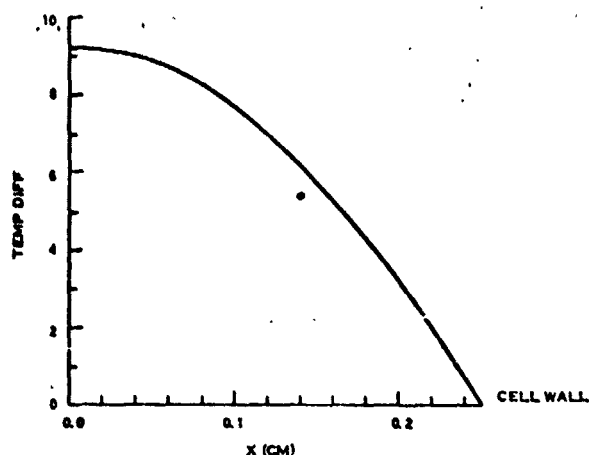


Fig. 9. Temperature gradient vs. distance from cell centerline for a field of 40 V/cm and a conductance of $8.7 \times 10^{-4} \Omega^{-1} \text{cm}^{-1}$. Cell thickness, 0.50 cm.

Curtain velocity profile: step 2

The linear velocity of the buffer curtain determines the residence time, t_r , of a particle in the electric field, and therefore determines, in part, the lateral displacement of the particle. In a constant temperature system, the velocity profile would be parabolic due only to viscous friction. However, viscosity decreases with increasing temperature (for a liquid) and since there is a distribution of temperature in the cell, a distortion of the parabolic flow profile results. It is important to know the buffer velocity at all points through the cell thickness, since a sample stream has a finite diameter and therefore particles at the outer edge of the stream move with a lower velocity than particles at the center. The slower parts of the stream have longer residence times and therefore experience different lateral electrophoretic displacements. This ultimately affects resolution.

The equation used to model the flow profile in the cell can be written as:

$$\frac{\partial}{\partial x} \left(\eta \frac{\partial V_b}{\partial x} \right) + \frac{dP}{dz} = 0 \quad (12)$$

where η is the viscosity of the buffer, dP/dz is the pressure gradient causing flow, V_b is the linear buffer velocity at x , a distance from the cell centerline.

Since it is not practical to measure dP/dz in a real system, this quantity must somehow be related to the volumetric flow-rate of the system, a quantity easily measured and controlled. This quantity dP/dz can be written as:

$$-\frac{dP}{dz} = \frac{\bar{\eta} 4F}{(4/3) ab^3 - (\delta/b) \sum_{n=0}^{\infty} N_n^{-3} \tanh N_n a} \quad (13)$$

$$N_n = (2n + 1)\pi/2b \quad (14)$$

where $\bar{\eta}$ is the average viscosity, a and b are one-half of the cell width and thickness, respectively, and F is the volumetric flow-rate²².

Figs. 10, 11 and 12 show the outputs for cells 0.07, 0.16 and 0.5 cm thick, respectively. The flow-rates in all three cases were adjusted to give comparable residence times in each cell.

The inputs to step 2 are: flow-rate, cell width, cell thickness and temperature points from the data file created in step 1 to calculate variations in viscosity.

Profile of electro-osmotic velocity: step 3

Electro-osmosis occurs normal to the direction of hydrodynamic flow. Since the cell is a closed system in the direction of electro-osmosis, this flow must be recirculating. Depending upon the sign and magnitude of the applied field and the ζ -potential at the wall, this electro-osmotic flow affects the lateral displacement of a particle undergoing electrophoresis. It is necessary to know the profile of this flow, so that a net horizontal displacement can be calculated for particles at various positions in the cell.

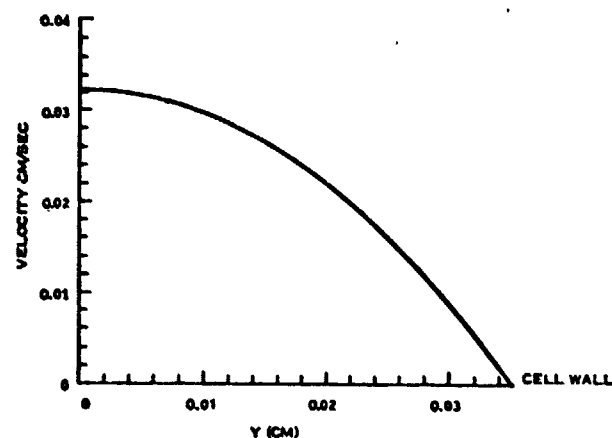


Fig. 10. Buffer curtain velocity vs. distance from cell centerline. Cell thickness, 0.07 cm.

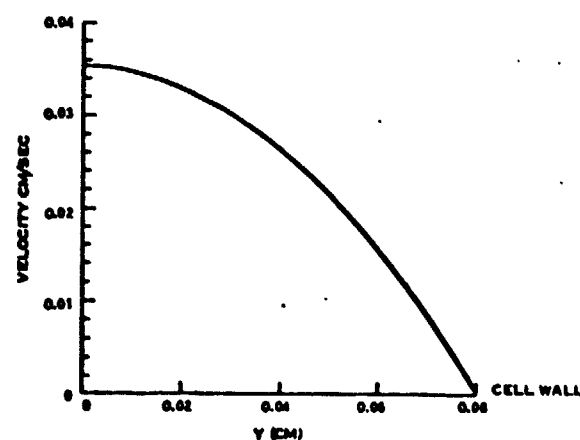


Fig. 11. Buffer curtain velocity vs. distance from cell centerline. Cell thickness, 0.16 cm

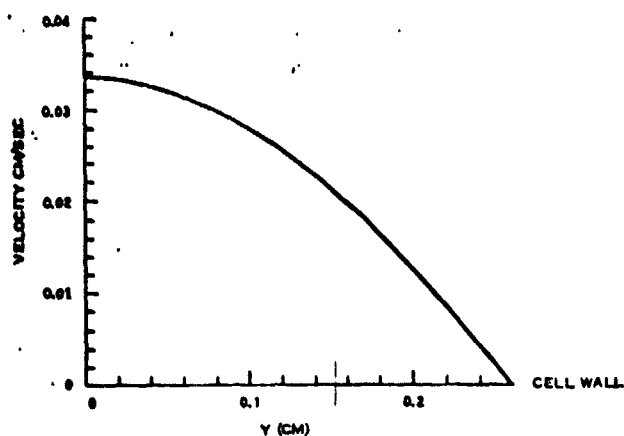


Fig. 12. Buffer curtain velocity vs. distance from cell centerline. Cell thickness, 0.50 cm.

The equation used to derive this profile is almost identical to that in step 2 and is written as:

$$\frac{\partial}{\partial x} \left(\eta \frac{\partial V_{eo}}{\partial x} \right) + F = 0 \quad (15)$$

where η is the viscosity, V_{eo} is the electro-osmotic velocity at distance x from the cell centerline and F is the driving force for electro-osmotic flow. An expression is needed to relate the ζ -potential of the wall to the force driving the fluid. If an average viscosity is assumed, then eqn. 15 becomes

$$-F = \bar{\eta} \frac{d^2 V_{eo}}{dx'^2} \quad (16)$$

and reduces to

$$V_{eo} = -\frac{Fx'^2}{2\bar{\eta}} + C_1 x' + C_2 \quad (17)$$

If the cell is described through its thickness as shown in Fig. 13, the boundary conditions are at $x' = 0$; $V_{eo} = V_w$. At $x' = s$, $V_{eo} = V_w$, so that eqn. 17 becomes:

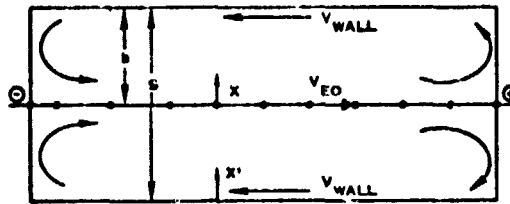
$$V_w = -\frac{F(0)^2}{2\bar{\eta}} + C_1(0) + C_2 \quad (18)$$

so that $C_2 = V_w$ and

$$V_w = -\frac{F(s)^2}{2\bar{\eta}} + C_1(s) + V_w \quad (19)$$

so that

$$C_1(s) - \frac{F(s)^2}{2\bar{\eta}} = 0 \quad (20)$$



$b = \text{HALF THICKNESS} = 1/2 s$
 $x = 0 \text{ AT CENTERLINE}$
 $y = 0 \text{ AT BOTTOM WALL; } y = s \text{ AT TOP WALL}$

Fig. 13. Closed-flow electro-osmosis.

and

$$C_1 = \frac{Fs}{2\bar{\eta}} \quad (21)$$

It follows that eqn. 15 is now transformed to

$$V_{eo(x')} = -\frac{Fx'^2}{2\bar{\eta}} + \frac{Fsx'}{2\bar{\eta}} + V_w \quad (22)$$

From Smoluchowski's equation for a rectangular cross section cell:

$$V_{eo(x)} = V_w - 6 V_w \left(\frac{x's - x'^2}{s^2} \right) \quad (23)$$

Setting equations 22 and 23 equal:

$$-6 V_w \left(\frac{x's - x'^2}{s^2} \right) = \frac{F}{2\bar{\eta}} (x's - x'^2) \quad (24)$$

$$-\frac{6 V_w}{s^2} = \frac{F}{2\bar{\eta}} \quad (25)$$

$$F = -\frac{12 V_w \bar{\eta}}{s^2} \quad (26)$$

Since $s = 2b$

$$F = -\frac{3 V_w \bar{\eta}}{b^2} \quad (27)$$

From eqn. 6

$$V_w = \frac{\zeta_w DE}{4\pi\eta} \quad (28)$$

Substituting into eqn. 27, the result is:

$$F = -\frac{3}{4} \frac{\zeta_w DE}{\pi b^2} \quad (29)$$

where D is dielectric constant, E is field, b is $1/2$ cell thickness and ζ_w is the ζ -potential. Now eqn. 15 can be written as:

$$\frac{3}{4} \frac{\zeta_w}{\pi b^2} = \frac{\partial}{\partial x} \left(\eta \frac{\partial V_{eo}}{\partial x} \right) \quad (30)$$

This last equation is the one used to calculate the final electro-osmotic velocity profile in the cell. Figs. 14, 15 and 16 are the results of these calculations for cells of 0.07, 0.16 and 0.5 cm thickness, respectively. Note that in each case there is a point at which V_{eo} is zero, and beyond that the flow direction reverses. This correlates well with the "real world" situation.

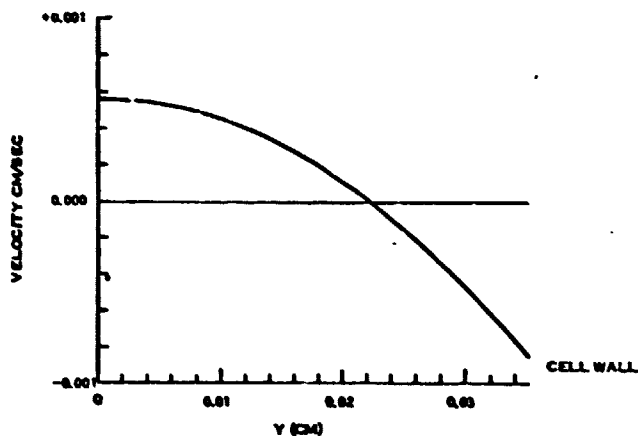


Fig. 14. Electro-osmotic velocity (V_{eo}) vs. distance from cell centerline for a field of 40 V/cm and a wall ζ -potential of 5 mV. Cell thickness, 0.07 cm.

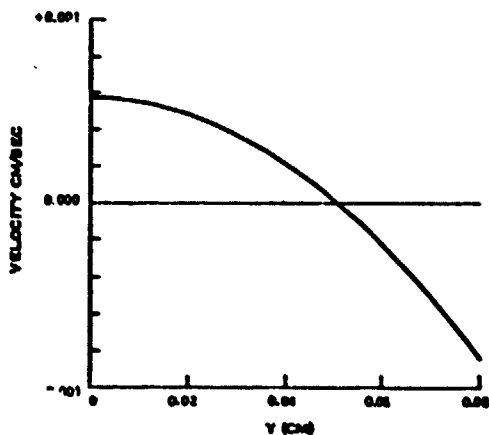


Fig. 15. Electro-osmotic velocity (V_{eo}) vs. distance from cell centerline for a field of 40 V/cm and a wall ζ -potential of 5 mV. Cell thickness, 0.16 cm.

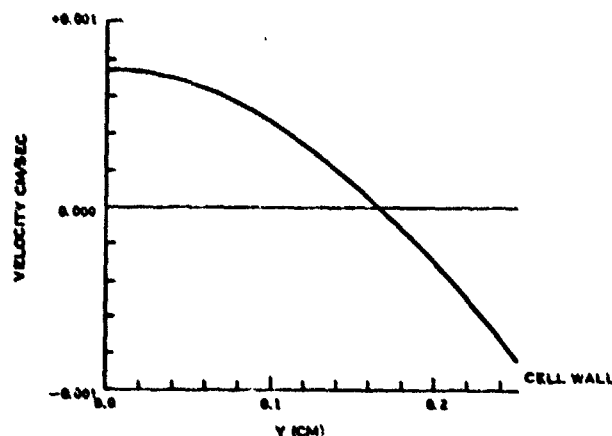


Fig. 16. Electro-osmotic velocity (V_{eo}) vs. distance from cell centerline for a field of 40 V/cm and a wall ζ -potential of 5 mV. Cell thickness, 0.50 cm.

Inputs to step 3 are: ζ -potential of the wall surface, dielectric constant of the buffer, the field gradient, 1/2 cell thickness and the temperature from the data file created in step 1 to calculate the variation in viscosity.

Diffusion effects and residence time (t_r): step 4

Depending on the kinds of particles in the sample stream and on the residence time within the field, diffusion effects may play an important role in the separation and resolution achieved. Diffusion will cause the sample stream to increase in diameter as it traverses the length of the cell. If the diffusion time is short compared to the residence time, sample particles will move into slower curtains and the residence times will increase. The effect of diffusing into a slower stream can be compared to a decelerating force and the increase in residence time can be calculated by using the following equation:

$$L = V_0 t_r - 1/2 A t_r^2 \quad (31)$$

where L is the length of the electrophoresis cell, V_0 is the initial velocity of a particle at the outer edge of the sample stream, A is the change in velocity with respect to time (due to diffusion) and t_r is the residence time.

The effect of diffusion can be related to eqn. 31 in the following manner: if the mean increase in sample diameter is expressed as²³

$$\Delta r = (6 D_1 t)^{1/2} \quad (32)$$

where D_1 is the diffusion coefficient and t is time, then the change in V with respect to time is given as

$$\frac{dV}{dt} = \left(\frac{3}{2} \frac{D_1}{t} \right)^{1/2} \quad (33)$$

Since $1r$ in this case corresponds to a change in x , the position through the thickness, it is possible to substitute dx/dt for $d1r/dt$. Now, acceleration or deceleration in this case is defined as

$$a = \frac{dV}{dt} \quad (34)$$

However, with a change of variables

$$a = \frac{dV}{dx} \frac{dx}{dt} \quad (35)$$

or

$$\frac{1}{2} a t^2 = \frac{1}{2} \frac{dV}{dx} \left(\frac{3D_1}{2t} \right)^{1/2} t^2 \quad (36)$$

from eqn. 35. Rearrangement brings

$$\frac{1}{2} a t^2 = \frac{1}{2} A t^{3/2} \quad (37)$$

where

$$A = \frac{dV}{dx} \left(\frac{3D_1}{2} \right)^{1/2} \quad (38)$$

Starting with the initial estimate of t , $\approx L/V_0$, a distance is calculated from eqn. 31 and compared to L , the actual length of travel. Using the Newton-Raphson routine an increment, DEL, is generated and added to the old residence time and the cycle begins again until the difference between the calculated distance and the actual distance meets the convergence criterion.

Table I contains the data obtained for three cells of thickness 0.07, 0.16 and 0.5 cm.

TABLE I
RESIDENCE TIME t , AT EDGE OF SAMPLE STREAM

In all cases $L = 10.16$ cm and $D_1 = 5 \times 10^{-9}$ cm²/sec.

| Parameter | Value | | |
|----------------------------|--------|-----------|-----------|
| Thickness (cm) | 0.07 | 0.16 | 0.50 |
| V_0 (cm/sec) | 0.008 | 0.030 | 0.033 |
| A (cm/sec ³) | -1.576 | -0.331 | -0.033 |
| t , 1st guess (sec) | 1163 | 335 | 307 |
| 1st iteration | 1679 | 338 | 308 |
| 2nd iteration | 1720 | converges | converges |
| 3rd iteration | 1721 | converges | converges |
| ΔX (cm) | 0.007 | 0.003 | 0.003 |

For particle remaining at the cell centerline, the residence time is simply the length, L , divided by the curtain velocity at $x = 0$.

The inputs to step 4 are: active cell length, buffer velocity at the edge of the sample stream ($bs/2$) (from step 2), the deceleration factor, A (from step 2), and the diffusion constants of the particles.

Total lateral displacement due to electrophoresis and electro-osmosis: step 5

The total lateral displacement of a particle in the field is the result of electrophoresis, electro-osmosis and residence time. In step 5, the net lateral velocities for the particles at points A and B in Fig. 3 are calculated. For the particle at point B, the net velocity is simply the sum of the electrophoretic and electro-osmotic velocities at $x = 0$. This sum times the residence time at $x = 0$ will yield S_0 , the lateral displacement at $x = 0$. Calculation of the similar term, S_x , for the particle at point A, involves integrating the electrophoretic velocity, V_{ep} , and the electro-osmotic velocity, V_{eo} , over the increase in sample diameter. S_x can be written as

$$S_x = \int_0^{t_r} \{V_{ep}(x_{(t)}) + V_{eo}(x_{(t)})\} dt \quad (39)$$

where $x_{(t)} = bs/2 + (6D_1 t)^{1/2} = bs/2 + \Delta x$ ($bs/2$ is the sample stream radius). The boundary conditions on $x_{(t)}$ are: when $t = 0$, $x_{(t)} = bs/2$ and when $t = t_r$, $x_{(t)} = bs/2 + \Delta x$, and from eqn. 35 $dx/dt = ([3/2] [D_1/t])^{1/2}$. The following substitution can be made:

$$dt = \frac{2(x - bs/2)}{6D_1} dx \quad (40)$$

From eqn. 39 and with a change in the limits of the integration, S_x can be written as:

$$S_x = \frac{1}{3D_1} \int_{bs/2}^{bs/2 + \Delta x} \{V_{ep}(x) + V_{eo}(x)\} \left\{x - \frac{bs}{2}\right\} dx \quad (41)$$

The inputs to step 5 are the diffusion constant of the particle, the sample radius increase and the residence time. The electro-osmotic velocity is taken as necessary from the data file created in step 3. The electrophoretic velocity is calculated from viscosity variations due to temperature gradient, and particle zeta potential.

Minimum resolution: step 6

Going back to Fig. 2, the separation resolution $l\mu$ can now be calculated from the data available:

$$l\mu = \Delta S + N + \Delta x' + bs/2 \quad (42)$$

where $l\mu$ is the minimum difference in sample mobility which will result in the complete separation of two sample components, N is the collection tube spacing,

$\Delta x'$ is the adjacent sample increase in radius due to diffusion and $bs/2$ is the original sample stream radius. ΔS , which is a measure of the sample distortion, is calculated from step 5 data by taking $\Delta S = S_x - S_0$. The absolute minimum is determined by two factors alone, the initial sample stream radius and the collection tube spacing, since it is conceivable to have a case where ΔS and $\Delta x'$ are both zero.

APPLICATION AND RESULTS*

Several realistic, yet hypothetical, cases were examined with the completed model. A sample containing four components was theorized. These components had ζ -potentials of 25, 29, 30 and 34 mV, corresponding to the mobilities measured for the fixed red blood cells of chicken, human A, human B and dog, respectively. In each case, the active cell width and length are 5.08×10.16 cm. The thickness was varied. The flow-rate through each cell was adjusted so that a particle at the centerline would have a residence time comparable to the other cases. The sample stream diameter, 0.06 cm, the wall ζ potential, 5 mV, and the field, 40 V/cm, were the same in all cases.

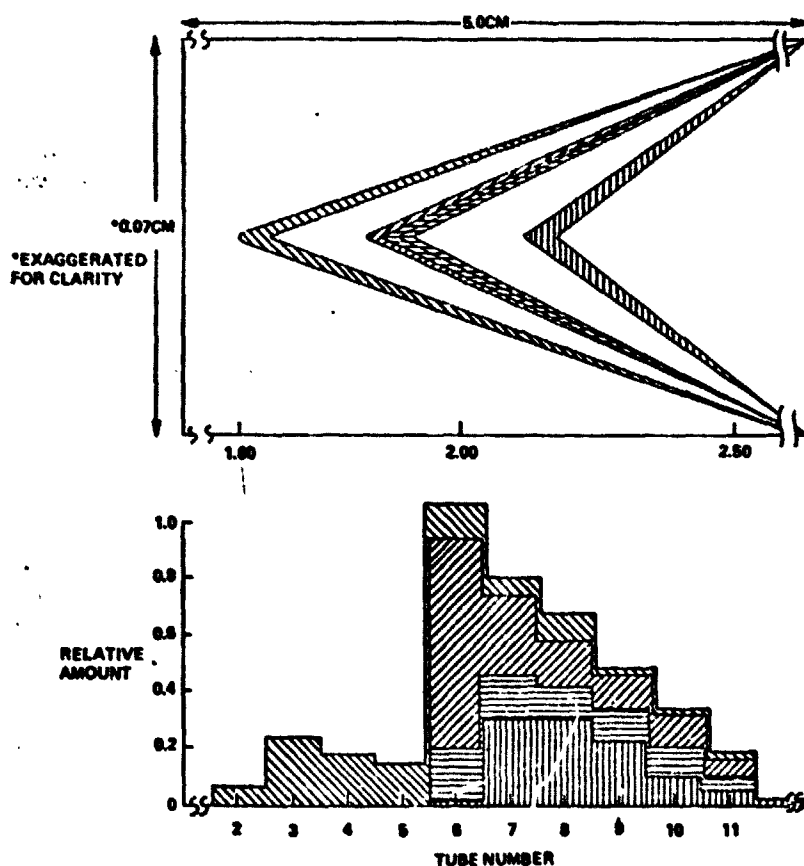


Fig. 17. Graphic illustration of separation and resolution for a 4-component mixture. Cell, 10×5 cm; thickness, 0.07 cm; sample diameter, 0.06 cm; field, 40 V/cm; wall potential, 5 mV; particle potential, 25, 29, 30 and 34 mV; centerline velocity, 0.032 cm/sec.

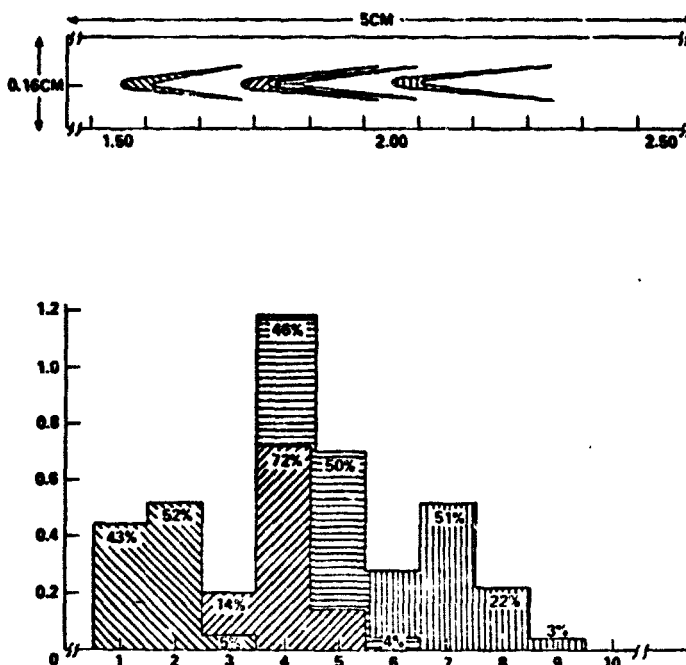


Fig. 18. Graphic illustration of separation and resolution for a 4-component mixture. Cell, 10 x 5 cm; thickness, 0.16 cm; sample diameter, 0.06 cm; field, 40 V/cm; wall potential, 5 mV; particle potential, 25, 29, 30 and 34 mV; centerline velocity, 0.035 cm/sec.

Figs. 17, 18 and 19 are the results of these calculations for cells of 0.07, 0.16 and 0.5 cm thickness, respectively.

The first case, a 0.07-cm thick cell (Fig. 17), is very close to the thickness of the electrophoresis cells described by Barrolier *et al.*⁴ and Hannig⁵. The effects of electro-osmosis and the buffer profile are profound. In addition, referring to Table I, the very long residence time for particles at the outer edge of the sample stream has caused the sample stream to diffuse to the cell walls even though a very small diffusion constant was used, *ca.* 10^{-9} . The result of moving to the wall is that the particles are now caught in the reverse flow caused by electro-osmosis and further remixing of the sample occurs. As can be seen from the collection graph, no separated material can be collected in any large amount.

The second case, a 0.16-cm cell (Fig. 18), is similar to equipment used in this laboratory. The crescent effect is still quite pronounced and it is still not possible to obtain a complete separation between any of the components.

The last case, 0.5 cm (Fig. 19), is a proposed "thick" zero g experiment cell. A cell of this dimension cannot sustain the resultant temperature gradient in a 1g environment without convection setting in rapidly. Although the crescent effect is still present, it is greatly reduced and it now becomes possible to obtain two components of 100% purity. Of the other two components, 95% of one component can be collected free of other material, while the fourth will contain some 4% (of the total amount) contamination.

In all three cases the ζ -potential of the wall was assumed to be 5 mV. This

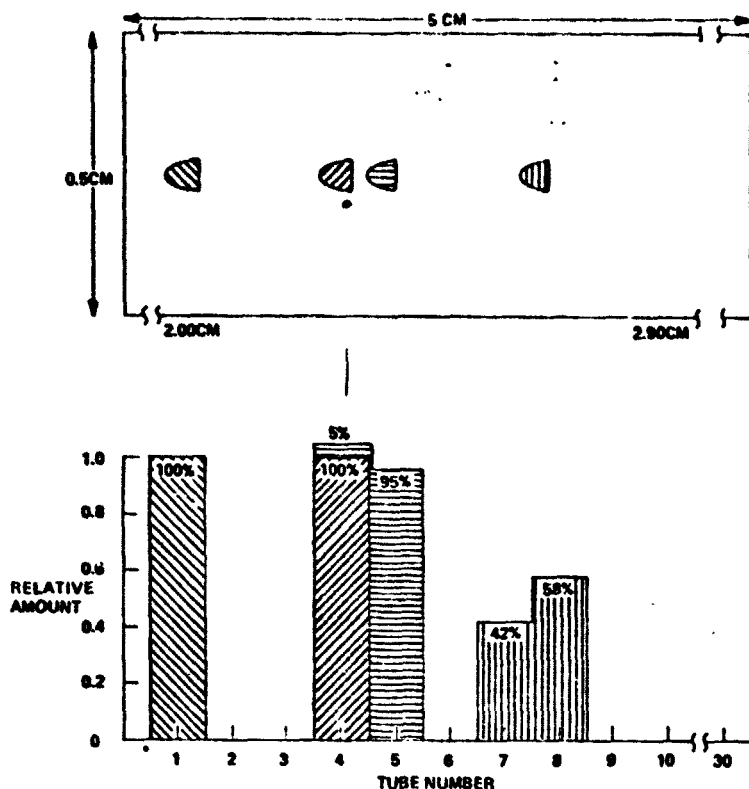


Fig. 19. Graphic illustration of separation and resolution for a 4-component mixture. Cell, 10×5 cm; thickness, 0.5 cm; sample diameter, 0.06 cm; field, 40 V/cm; wall potential, 5 mV, particle potential, 25, 29, 30 and 34 mV; centerline velocity, 0.033 cm/sec.

would result in very little electro-osmotic flow and the bulk of the crescent effect is due to the buffer flow profile.

The crescents, in these cases, point from right to left, indicating that particles at the outer edge of the sample stream had more lateral electrophoretic displacement due to longer residence times.

If the wall ζ -potential is changed, for example from 5 to 50 mV, by some treatment of the wall surface, the results would look like Fig. 20. The other parameters are unchanged from case 3. The crescent is now pointing from left to right due to the increased influence of electro-osmosis. Overall, the electro-osmotic velocity at any point is greater than the electrophoretic velocity. As a result, the entire sample band moves farther than previously and particles near the centerline move farther than others in the stream.

Table II lists some data pertaining to these examples. The distortion, ΔS , is given and the separation resolution, $\Delta\mu$, is given in both $\mu\text{m} \cdot \text{cm} \cdot \text{V}^{-1} \text{sec}^{-1}$ and in mV.

The first two examples were modeled after existing equipment to check the reliability of the predictions made with the mathematical model. The last two cases are, at present, unable to be verified experimentally, since they require a "zero g" environment. An electrophoresis cell of the dimensions stated for cases 3 and 4 has

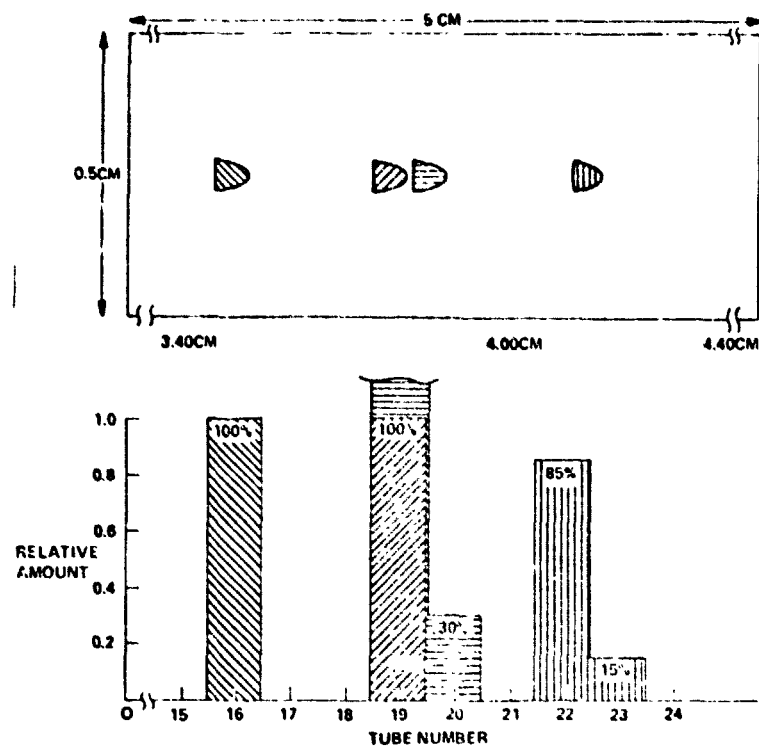


Fig. 20. Effect of a change in the wall ζ -potential. Conditions are the same as in Fig. 19 except the wall ζ -potential has been increased by a factor of 10.

TABLE II
HYPOTHETICAL ELECTROPHORETIC SEPARATIONS

$\Delta S = \text{cm}$

$\Delta\mu = \mu\text{m} \cdot \text{cm} \cdot \text{V}^{-1} \cdot \text{sec}^{-1}$

| Separation parameters | ζ -potential | | | | Other parameters |
|----------------------------|--------------------|-------|-------|--------|--|
| | 25 mV | 29 mV | 30 mV | 34 mV | |
| ΔS | 4.96 | 6.00 | 6.27 | 7.31 | 0.07 cm thick; $\zeta_w = 5 \text{ mV}$ |
| $\Delta\mu$ | 4.08 | 4.89 | 5.11 | 5.94 | |
| $\Delta\zeta \text{ (mV)}$ | 78.77 | 94.42 | 98.66 | 114.69 | |
| ΔS | 0.19 | 0.23 | 0.24 | 0.27 | 0.16 cm thick; $\zeta_w = 5 \text{ mV}$ |
| $\Delta\mu$ | 0.28 | 0.31 | 0.32 | 0.35 | |
| $\Delta\zeta \text{ (mV)}$ | 5.41 | 5.98 | 6.17 | 6.75 | |
| ΔS | 0.024 | 0.026 | 0.027 | 0.032 | 0.50 cm thick; $\zeta_w = 5 \text{ mV}$ |
| $\Delta\mu$ | 0.137 | 0.137 | 0.137 | 0.137 | |
| $\Delta\zeta \text{ (mV)}$ | 2.645 | 2.645 | 2.645 | 2.645 | |
| ΔS | 0.034 | 0.029 | 0.028 | 0.024 | 0.50 cm thick; $\zeta_w = 50 \text{ mV}$ |
| $\Delta\mu$ | 0.138 | 0.137 | 0.137 | 0.137 | |
| $\Delta\zeta \text{ (mV)}$ | 2.664 | 2.645 | 2.645 | 2.645 | |

been constructed and some ground-based data were obtained. These data were then compared to data for thinner cells and then extrapolated to thicker cells. Fig. 21 relates these data, power density, residence time and cell thickness to an arbitrary stability standard. This standard was defined as: an undisturbed flow of neutral density polymer latex for a minimum of 3 min with the field applied. The result is the curved surface shown in Fig. 21. In a 1g environment stable operation of the system will occur only for points lying below the surface. Operation at the surface or above it suggests a reduction in the gravity field to decrease convection.

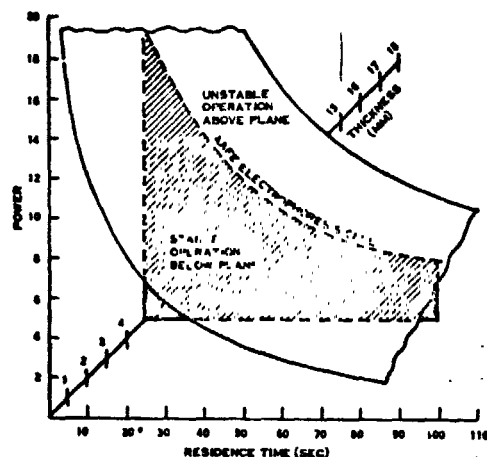


Fig. 21. Experimentally determined regions of stable and unstable operation of an electrophoresis cell as a function of the power (in W) and the residence time for a 0.50-cm thick cell with other conditions as indicated.

Sedimentation of sample at high concentrations is another problem experienced by early researchers. In a preparative system sample throughput would be of prime concern. One solution to the sedimentation problem is to operate the system vertically. However, this orientation tends to maximize convection. Again, a reduced gravity field would serve to overcome these problems. Data were obtained for existing equipment and extrapolated to thicker cells. Fig. 22 shows throughput in g/h versus cell thickness for two kinds of sample streams. The first kind of stream is very thin in width, less than the inside diameter of a collection tube, and this width is kept constant. The height of the stream varies with the cell thickness. This kind of stream would be used when resolution of components is the main concern, recalling the criterion for separation resolution. The second kind of sample stream is round in cross section and its diameter is ca. 80% of the cell thickness. This kind of stream would be used when high throughput of sample is the objective. Comparing the two kinds of streams, at 10% sample concentration in a 10-mm thick hypothetical cell, the increase in throughput from the rectangular cross section to the round cross section would be greater than a factor of 20.

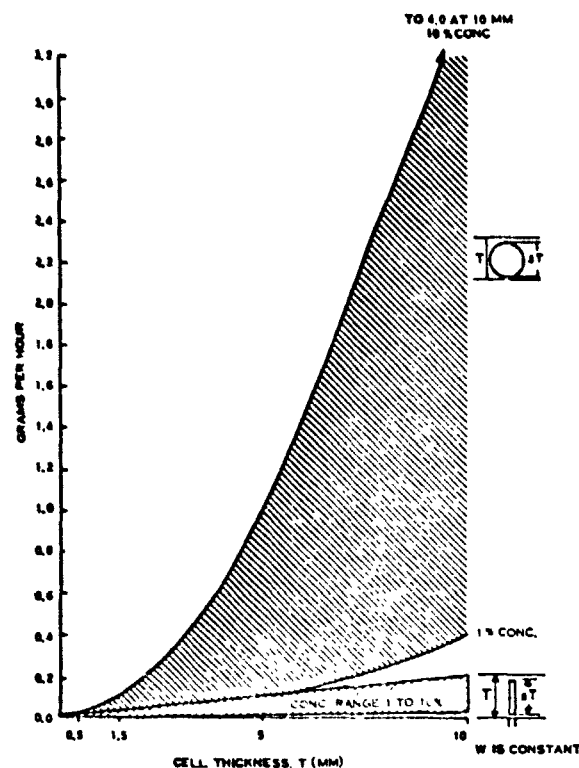


Fig. 22 Throughput vs. cell thickness. Lower curves represent sample concentrations for high resolution. Upper curves represent sample concentrations for maximum throughput.

CONCLUSIONS

A mathematical model of an electrophoresis cell operating under conditions of no convection and no sedimentation has been assembled. Through the use of computers, the complex interactions of the various parameters are able to be modeled realistically. The temperature gradient is probably the most important factor affecting a given separation, since this directly affects the buffer curtain profile and the electro-osmotic profile through the temperature dependence of the fluid viscosity. For a cell of given length and width, the thickness has the most profound effect on the temperature gradient since the heat transfer occurs through this dimension. For temperature stability a thinner cell is advisable. However, the buffer profile and electro-osmotic profiles are adversely affected in thinner cells except under special circumstances^{7,8}. Therefore, a trade-off must be made between temperature and the two flows for systems operating in 1g environments. If the problem of convection in thick cell systems is eliminated by operation in a zero g environment the upper limit for cell thickness is governed by the maximum temperature the sample can withstand. In this way, the buffer curtain profile and electro-osmotic profile are kept as flat as possible.

Sedimentation of sample at high concentrations is another problem which plagues terrestrial free flow electrophoresis systems. By operating the system in zero g, the sedimentation of sample is negligible, allowing higher throughput.

When the inputs to the model correspond to existing equipment, the theoretical results of the model compare favorably with actual data. These results provide the basis for predicting the separation, separation resolution and throughput for thick cell systems to be operated in a zero g environment.

ACKNOWLEDGEMENT

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ELECTROPHORESIS FOR BIOLOGICAL PRODUCTION

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Abstract

Preparative electrophoresis may provide a unique method for meeting ever more stringent purity requirements. Prolonged near zero gravity in space may permit the operation of preparative electrophoresis equipment with 100 times greater throughput than is currently available. Some experiments with Influenza Virus Antigen, Erythropoietin and Antihemophilic Factor, along with process and economic projections, will be briefly reviewed.

Introduction

The idea of preparing biologicals of improved purity and specificity in space has both great technical and economic basis. It could become a multi-billion dollar business and be an important use for the STS. There is however a great deal of research and development to perform first.

This paper reviews some of the early work; from the initiation of the idea through some flight demonstrations and on to the current development of a sounding rocket experimental unit and some ground based separations work. A brief concluding section then outlines some of the projections for possible future preparative electrophoresis in space.

Early History of Electrophoresis in Space

Electrophoresis has been widely used for several decades, primarily based on the work of Tiselius, for analysis of biological materials. There are now an estimated 30,000 research and analysis personnel who utilize the technique in the U.S. alone and several hundred technical papers are based on this work annually.

Unlike other analysis and process techniques it has not been possible however to scale up the electrophoretic analytical technique to provide a truly preparative scale of operation. This is primarily due to gravity induced convection and sedimentation which can be sufficiently counteracted by such approaches as the use of gels, orientation, cooling, and small dimensions in the case of analytical devices, but are too restrictive to permit scaling up to an economical preparative level.

Thus in a "brain storming" type of discussion on space processing ideas among staff members of the Wyeth Laboratories and General Electric Space Sciences Laboratory in the spring of 1969, preparative scale electrophoresis was suggested among the several ideas at that meeting. Several possible product examples such as vaccines, hormones, enzymes, and cells were suggested by Wyeth as well as other organizations over the next year or so.

About a year later, specific R&D work was initiated on the idea and almost immediately an opportunity arose to have a flight demonstration on Apollo 14. In about four months, we then designed and developed a small flight demonstration shown in Figures 1 and 2.

Samples of salmon sperm DNA, hemoglobin, and a mixture of red and blue dye were chosen to represent a broad range of molecular weights and to demonstrate electrophoretic mobility under microgravity conditions. Only the red and blue dye were expected to be, and were, electrophoretically separated.

Although the results were not up to our high expectations, the red and blue dyes did separate but the photography did not provide clear pictures. The biologicals were destroyed, apparently by bacteria, during the four month flight and quarantine period, but the engineering aspects of the unit were excellent and were reused on later flights.

A second flight demonstration was then scheduled on Apollo 16 with again about four months to develop it. Steps were taken to overcome the problems of the Apollo 14 flight, namely: a tripod and lens extension tube system was provided to improve the photography (Figure 3) and PSL (polystyrene latex) was suggested by the USRA (University Space Research Association) as a more stable non-biological sample.

Along with the choice of PSL as the sample, ground based work using sucrose solution density gradients was suggested and used to indicate (Figure 4) and define the separation of the 0.2 and 0.8 micron PSL which was flown as a mixture in the upper tube and individually in the bottom and middle tubes, respectively, of the apparatus.

An example of the results of the Apollo 16 demonstration is shown in Figure 5 with a ground based view for comparison. A clear indication of the possible improvements in electrophoretic separation performed in space is indicated even though electroosmosis and some bubbles are also indicated. The latter two problems warrant some brief mention.

The bubbles were, we now believe, caused by the permeability of the silicone tubing - especially when it is subjected to a rapid external depressurization as was the custom on Apollo flights. During the development of the Apollo 16 unit there had been some indication of stress corrosion problems with the Lexan used to fabricate the electrophoresis cells. This was primarily due to the use of thin wall sections for greater transparency as compared to the more massive monolithic machined block used for Apollo 14. While the design was indeed demanding of the full capabilities of the Lexan, it was the best choice of transparent plastic and probably not the source of fluid leaks that permitted bubbles to form.

The electroosmosis is the result of the high zeta potential on the walls of the electrophoresis chambers. At that time there was no low zeta potential coating available which would adhere adequately to the chamber walls, and ground based tests in sucrose density gradients indicated no benefit from the use of a collodion coating as compared to leaving the Lexan uncoated. Since applying a coating may have been detrimental to the Lexan from the stress-corrosion standpoint, it was decided to leave it uncoated. The problem then is one of either not being able to translate the density gradient work to the flight demonstration unit or in not being able to obtain ground based results from the flight demonstration unit that would show the electroosmosis problem.

Happily, the remaining problems with these two flight demonstrations have been overcome in the more major experiment MA-011 (which on the other hand had some other difficulties which are being assessed and reported separately). The MA-011 also again made use of the phase separators and small peristaltic pump plus other technologies from Apollo 14 and 16. Thus it appears that while each flight has corrected the deficiencies of the previous flight, new problems have arisen by virtue of the changes made in

the demonstration materials, or design, or equipment, as each unit was being hurriedly developed for a singular flight opportunity.

It is therefore highly satisfying to see the sounding rocket and space shuttle flight schedules and the possibility for repeated flights of an experiment until it is satisfactory for as long as warranted.

Preparative Electrophoresis

Equipment - With the completion of the efficiently satisfactory Apollo 14 and 16 flights, our attention was turned toward meeting the original and still desirable goal of developing a truly preparative unit for space experiments. We chose the continuous flow type of unit as offering the greatest ease of inserting and removing samples and sample fractions. While the engineering of such a unit requires ingenuity, it is not extremely difficult and the basic idea for the electrophoresis cell is to simply make it thicker than the 0.5 to 1.5 mm commonly used for such units on earth. It was estimated that the cell in such a unit for space could be as much as 8-10 mm thick and provide an improvement by a factor of about 5-10 in resolution or an improvement of about 80-100 in throughput. This much greater performance is simply due to being able to scale up the thickness without gravity induced convection and to increase the sample concentration without sedimentation problems.

This has now been demonstrated with a 4 mm thick cell, at least partially, on the ASTP-MA-014 experiment by Hannig. A similar unit has also been developed in our laboratory for sounding rocket usage. It is shown in Figure 6 as currently equipped with a camera for data acquisition. Other work is now underway on modifications to permit collecting up to 50 fractions of sample and to detect them by a U. V. scanner system. These are being done on a schedule to permit a flight test in late 1971. The cell is 5 mm thick by 5 cm wide and has a 10 cm long electrode section. It is supplied with approximately 4°C buffer, coolant, and samples by the use of a passive refrigerant system. The possible operating conditions such as flow rates, volts/cm across the cell, etc. are very broad and can be adjusted over several decades by a choice of gear ratios on the pumps and by plug-in power supplies, as well as by "fine tuning" electrically up to a short time before flight.

Math Modeling and Computer Simulation - Prior to designing the current sounding rocket unit, a math model was prepared both to aid the design and to predict the performance of thick cell electrophoretic separators in space. A separate publication is in preparation on this work so it will only be briefly reviewed here. Figure 7 shows the factors considered in the math model along with indications of which are controllable. As compared to previous efforts to describe the operation of a continuous electrophoresis cell, the parameters in our model are allowed to interact and are calculated primarily as to their effect on resolution and secondarily, throughput. Some typical results for a realistic although hypothetical separation of four samples (as defined by zeta potential and other conditions in each of three different thickness flow cells) are shown in Figures 8, 9, and 10.

Extensive ground based testing in prototype electrophoresis cells built for the previously described sounding rocket, as well as with other electrophoresis units, has generally corroborated the calculations for at least low levels of power. A plot of the power versus resident time (as a measure of flow rate) and thickness for stable and unstable conditions (hydraulically) is presented in Figure 11. Unfortunately, gravitational convection induced by the joule heating in these thick cells occurs at levels of power (10-15 watts) which are an order of magnitude below the power levels useful for separation.

Therefore, while the calculations and ground based tests at low levels are in good agreement, the more realistic level tests will have to be accomplished in space under micro-gravity conditions.

Biological Tests - Ground based tests for both the development of equipment and for establishing operating conditions for biologicals are being accomplished in a commercially available unit shown in Figure 12. It is a Beckman CPE II which is well designed for model studies and to which we are making additions and changes for more easily handling biologicals. These include fused silica windows and a U.V. scanner, for example. Studies ranging from single experiments, so far, to about 20 experiments, plus numerous calibration runs with PSL, have been undertaken with each of various biologicals including:

- Hepatitis Vaccine
- Sperm
- Lymphocytes
- AHF
- Erythropoietin
- Influenza Virus Antigen

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The results are generally encouraging but not necessarily easily achieved nor sufficiently complete. Considerably more effort has to be expended in this area before flight tests since it seems unlikely that a space flight test will accomplish a separation that has not at least been shown to be feasible on earth. Examples of some of the separations studied and results obtained are shown in Figures 13-15.

Projections for the Future - Contacts in numerous pharmaceutical houses indicate that, while indeed cells of various types and sources are an intriguing problem for separation science, numerous hormones, enzymes, blood and urinary source materials, and vaccines need or would be benefited by a great deal of improvement in purity. The practical limitation on the use of electrophoresis to prepare these products in sufficient purity to be of value is throughput efficiency. While absolute purity is required in certain cases, many products are only needed in more concentrated form and therefore resolution is often a subjective parameter which can perhaps be traded off against throughput. An estimate of the throughput in grams/hour versus cell thickness for two cases is shown in Figure 16. The upper right hand area of the figure depicts a high throughput case, i.e. for a case where sufficient resolution is easily obtainable and the extra capacity of the equipment can be utilized for throughput. The lower portion of the figure depicts a situation where resolution is to be stressed. In each case a range of 1-10% sample concentration is shown which should be compared with typical ground based practice of using about 0.1 to 0.5%. In any case, some 2 to 3 orders of magnitude improvement in throughput are predictable. This is far beyond the degree of improvement for which one might consider simply duplicating the ground based facilities when greater throughput is needed even if resolution were satisfactory.

Economic Predictions - Three general areas of potential payoff for this work are foreseen. First is the possibility of the research and development being beneficial to ground based electrophoresis equipment and techniques. Secondly is the possibility of preparing more specific strains or products in space which can then be used to culture and produce greater quantities of particular products on earth. Thirdly, when the first two or other approaches are insufficient, products may actually be produced in space.

Examples of the first two approaches already being productive are available. Improved electrophoresis equipment and coatings with nearly zero zeta potential are now available

and are examples of the first area of benefits. Increased yield of Urokinase through the improved separation of fetal kidney cells on the Apollo Soyuz Test Project flight in the summer of 1976 is an early indication of a potential benefit in the second area. Preliminary examples of the third area must await further work but may well come from current projects for the sounding rocket and early shuttle flights.

Several products could potentially benefit from these capabilities and further work is recommended to establish the necessary protocols and reference data on which to base flight tests.

The human value of more effective biologicals is of course impossible to measure. The preparation of purer erythropoietin could free some 15,000 U.S. renal failure patients from repeated blood transfusions. Thus humanitarian and societal motivation in this area is unusually high, and even greater than the basic economic value. Some simple projections for space processing of biologicals can be made based on certain assumptions.

It is presumed first that for efficiency and economy, as much of the processing as possible will be done here on Earth. Then, only a reasonably pure concentrate will be taken to space for one more, or perhaps a few, processing steps. In addition, the large quantities of water normally used in biological processing are presumed to be recoverable and reusable in space so that this commodity will not need to be completely resupplied from Earth for each product. Finally, however, the general rule that each biological product should be prepared in isolation from other products in order to avoid cross contamination is likely to be necessary. This may necessitate some special scheduling, but should not create any insurmountable problems.

Vaccines are the best defined available product on which to base projections for the future. In the U.S., some 60 million doses of vaccine are used annually. If we utilize the World Health Organization's estimates of World population in 1990-2000 as 5 billion and assume the same rate of vaccine applications world-wide as is now current in the U.S., we project the need for about 1.5 billion doses of vaccine per year. Using a conservative average number of 100,000 doses per gram of active ingredient, we calculate the need for 15,000 grams of active ingredients per year. Many currently used and very fine biological products are at best however quite dilute or impure (but not necessarily with harmful impurities). The purity may range from less than 1% to about 50%. This is assumed to be the starting material for a space purification operation. Therefore, the weight of starting material could range from 2 to 100 times the 15,000 gram final product weight derived above. Assuming a conservative average of 50, it is expected that some 750 Kg of partially purified vaccines might be used as the starting materials. In addition, some several hundred kilograms of water would be required. While vaccines generally cost about 20¢ per unit to produce, some examples of higher costs for greater specificity indicate that \$1.00 per unit may be an acceptable value. This then indicates a \$1.5 billion dollar activity in vaccines alone, a fraction of which may require space operations.

The processing of some other biological products such as cells and the blood derivative in space while less specifically calculable could easily exceed the estimates for vaccines by up to an order of magnitude in volume and value.

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Acknowledgement

The support and encouragement for much of this work by NASA through several contracts is appreciated and acknowledged. In addition, major roles in various aspects of this work have been filled by several associates especially Dr. R. N. Griffin, R. J. Locker, Dr. J. Giannovario, and Frank Cosmi, as well as others too numerous to mention. It is a pleasure to acknowledge the work of all of them.

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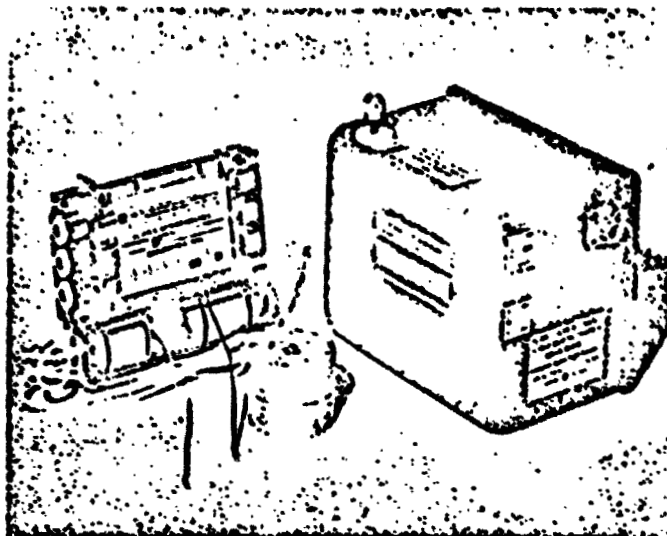


Figure 1. Apollo 14 Fluid Electrophoresis Demonstration Unit on right with some of the major components on left. These include the three electrophoresis cells machined in a monolithic block of Lexan, with phase separators below and peristaltic pump in center of photo. Overall dimensions of experiment are about 4" x 5" x 7" plus appurtenances.

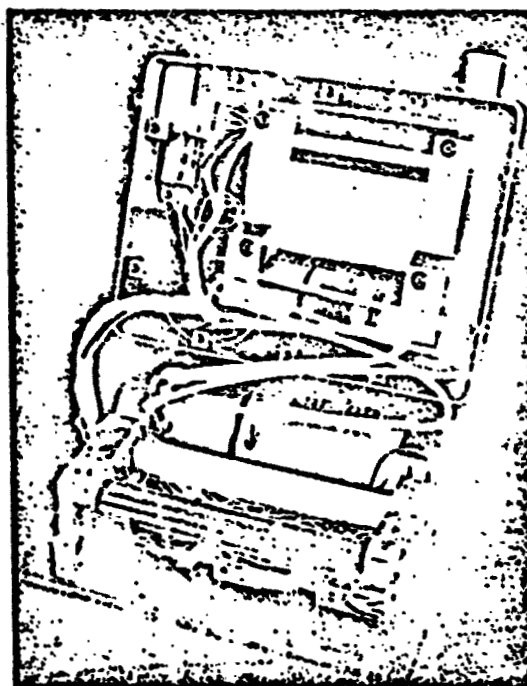


Figure 2. Apollo 14 Fluid Electrophoresis Demonstration Unit in opened position showing back view of electrophoresis cell in upper portion of box with phase separators and peristaltic pump in middle and fluorescent lamps plus potted electronics in lower area.

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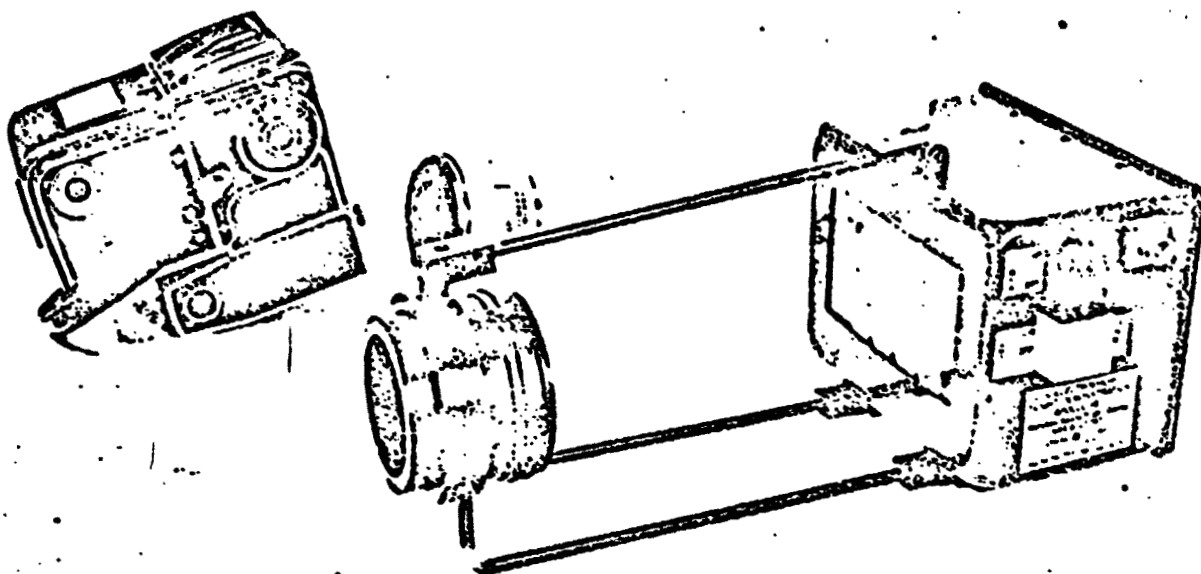
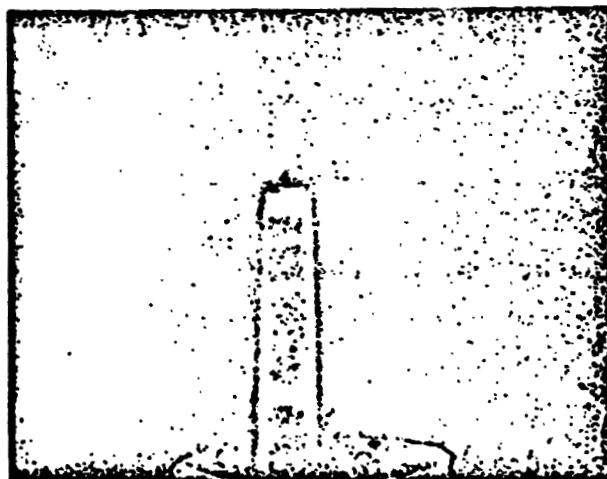


Figure 3. Apollo 16 Fluid Electrophoresis Demonstration. Note larger window, instruments, and camer tripod arrangement to improve data acquisition.



Electrophoretic Separation of Polystyrene Latex in a Sucrose Density Gradient

Figure 4. Electrophoretic Separation of 0.2 and 0.8 micron Polystyrene Latex in a Sucrose Density Gradient after 40 minutes during which time the leading band (0.8 μ) traveled 8 cm and the trailing band 5.6 cm using a 0.085 M Borate buffer of pH 8.5.

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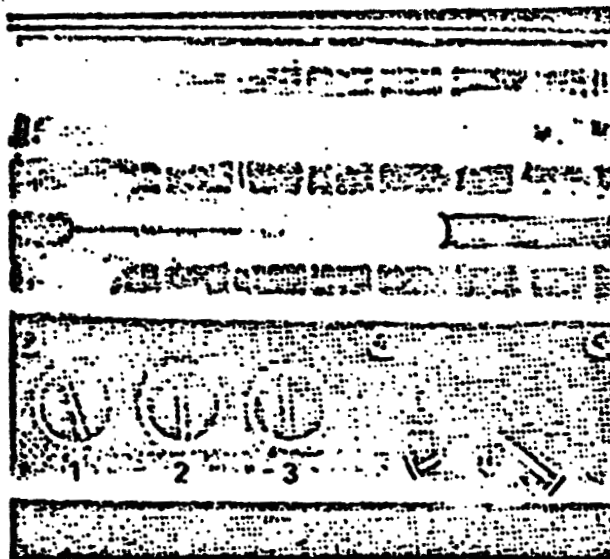
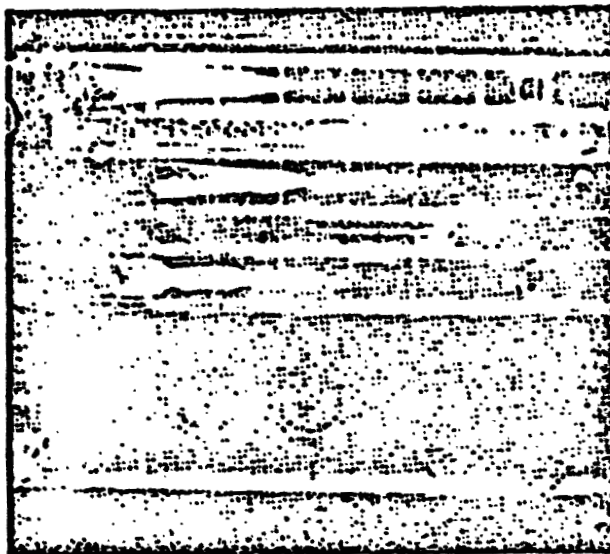
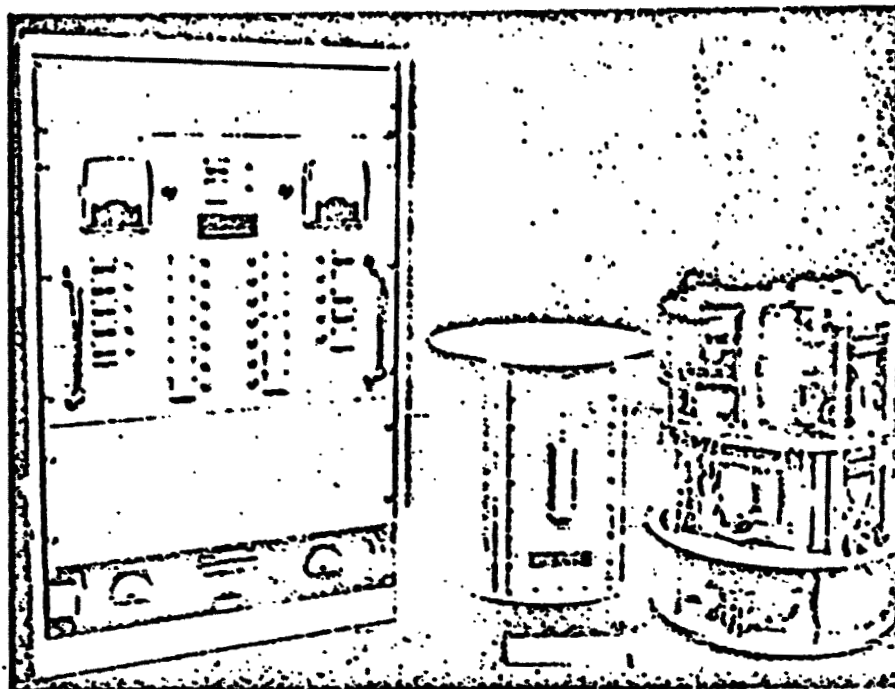


Figure 5. Flight (upper) and Ground Based (lower) results from Apollo 16 Fluid Electrophoresis demonstration. The flight results clearly show the benefit of reduced gravity on the electrophoretic mobility at 30 V/cm in borate buffer of a mixture of 0.2 and 0.8 μ polystyrene latex in the upper tube, 0.3 micron PSL in the middle and 0.2 micron PSL in the lower tube. Equivalent samples in the lower, ground based, photo show the detrimental effects of gravity induced convection and sedimentation.



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Figure 6. NASA-AAFE (Advanced Applications Flight Experiment) free flow electrophoretic separator (at right) under development for use on a sounding rocket. The upper enclosure with an access door for installing the sample, servicing the camera and setting experiment conditions immediately prior to flight is shown in the middle with the test and control panel including power supply at left.

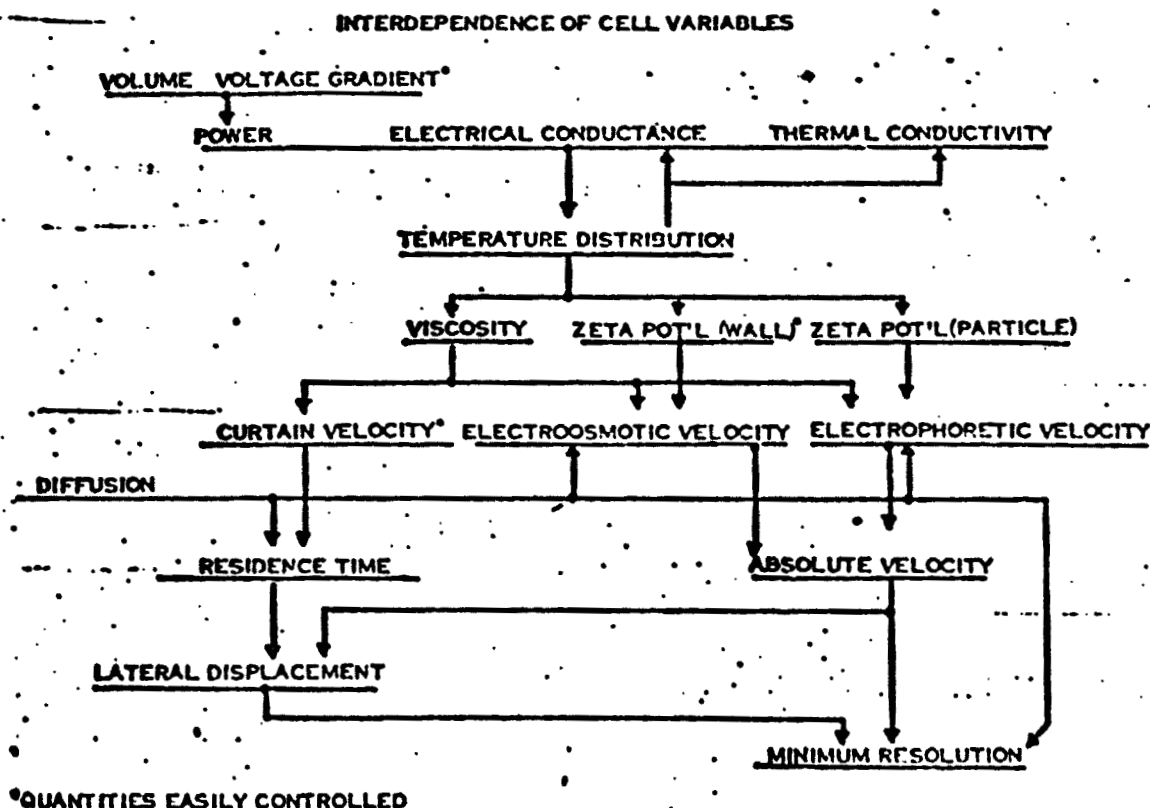
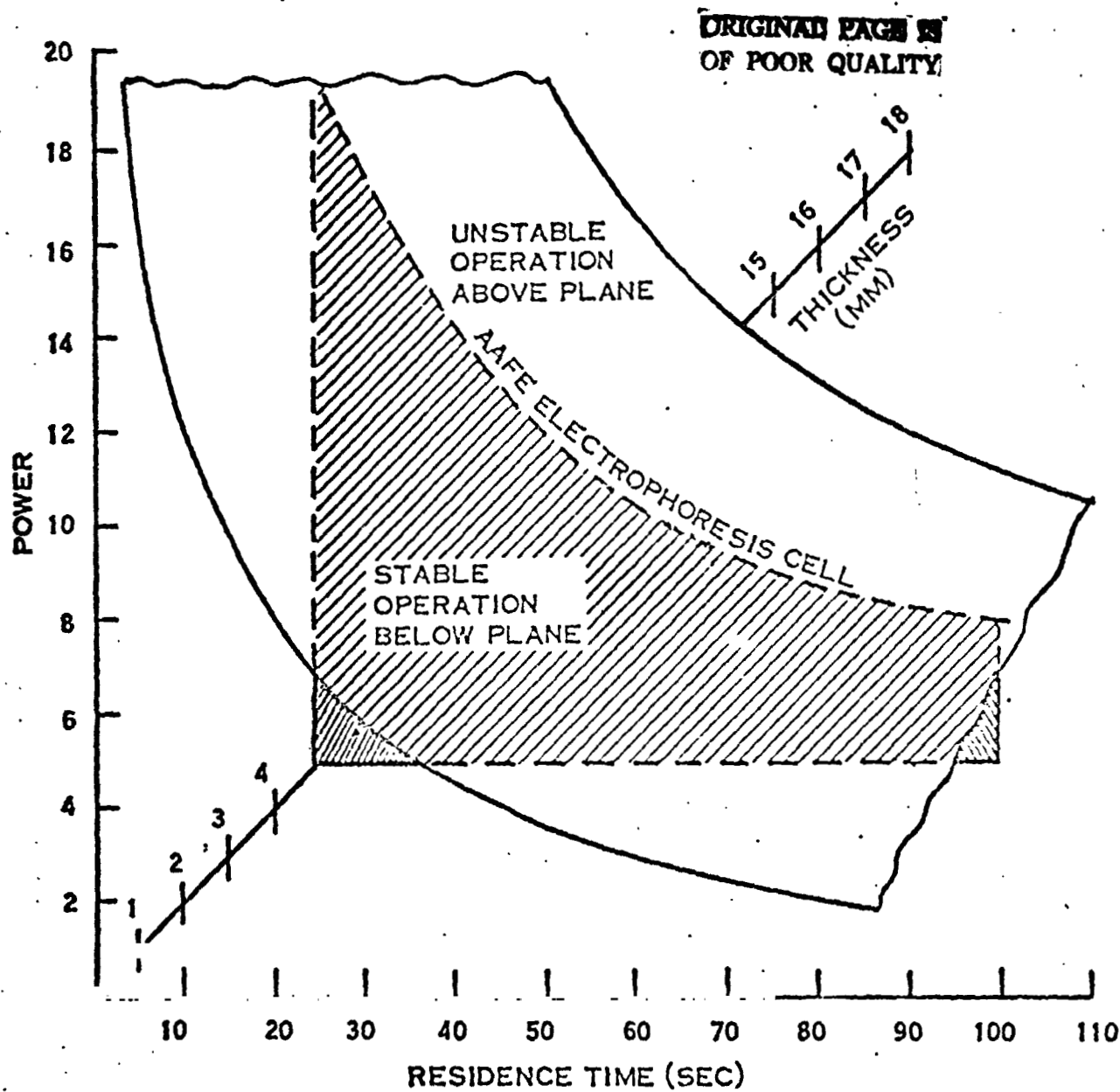


Figure 7. The major features of a computerized math model of a free flow electrophoretic separator.



ACTIVE AREA 5CM X 10CM
 SAMPLE: NEUTRAL DENSITY POLYSTYRENE LATEX
 ORIENTATION 20° FROM VERTICAL

Figure 11. Experimentally determined regions of stable and unstable operation of the NASA-AAFE (See Figure 6 caption) electrophoresis cell as a function of power, and residence time for 5 mm thick cell with other conditions estimated.

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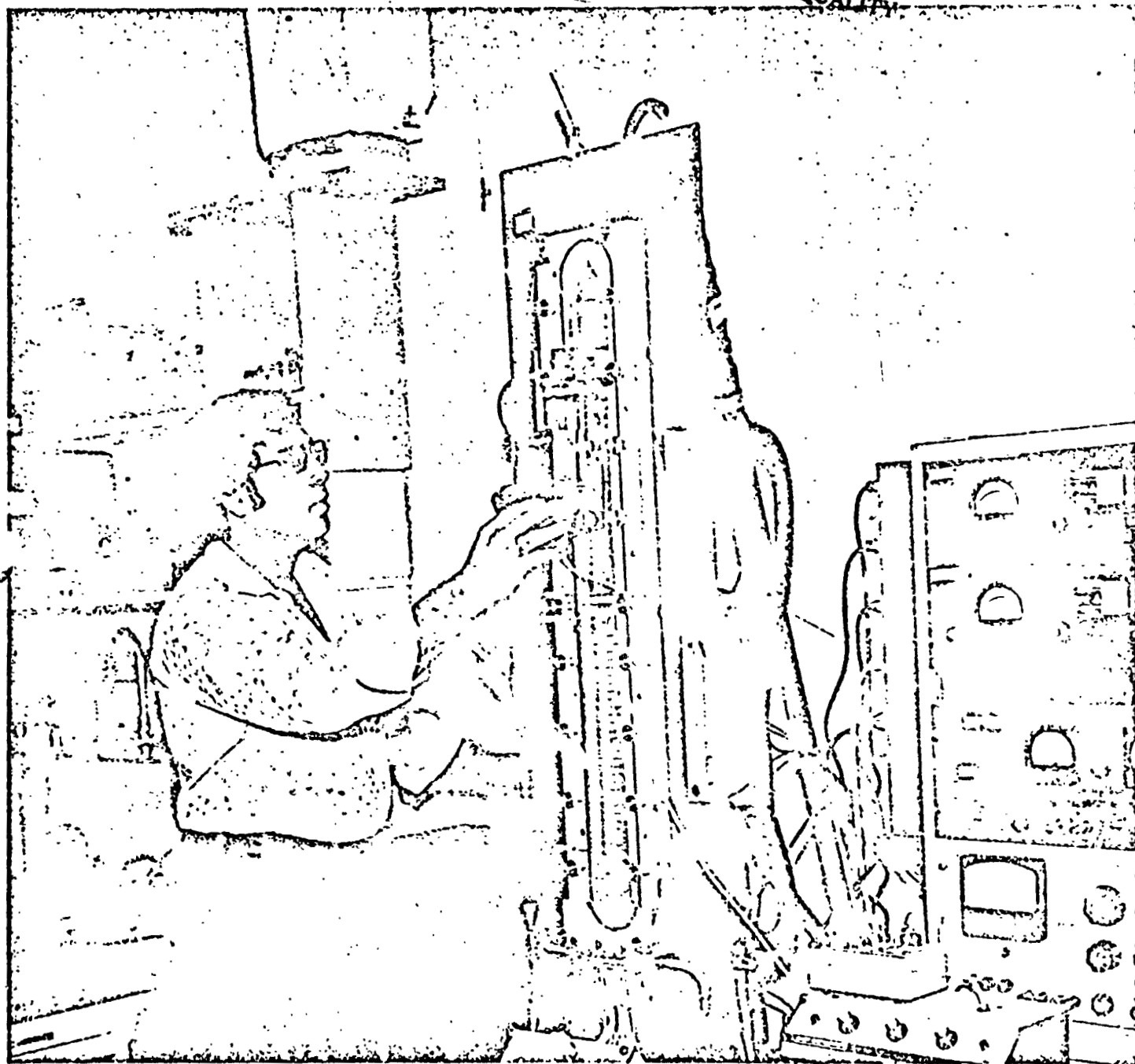


Figure 12. Modern laboratory continuous flow electrophoretic separator.

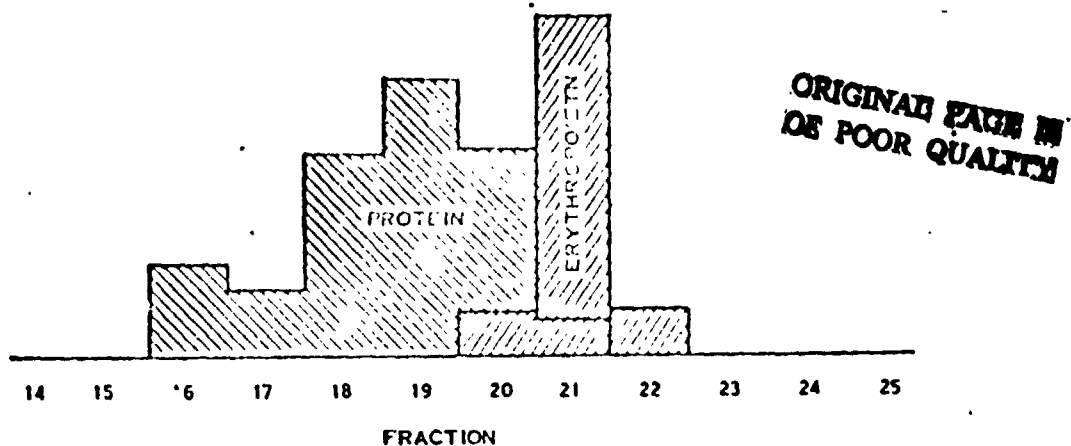


Figure 13. Partial concentration of Erythropoietin from protein by electrophoresis.

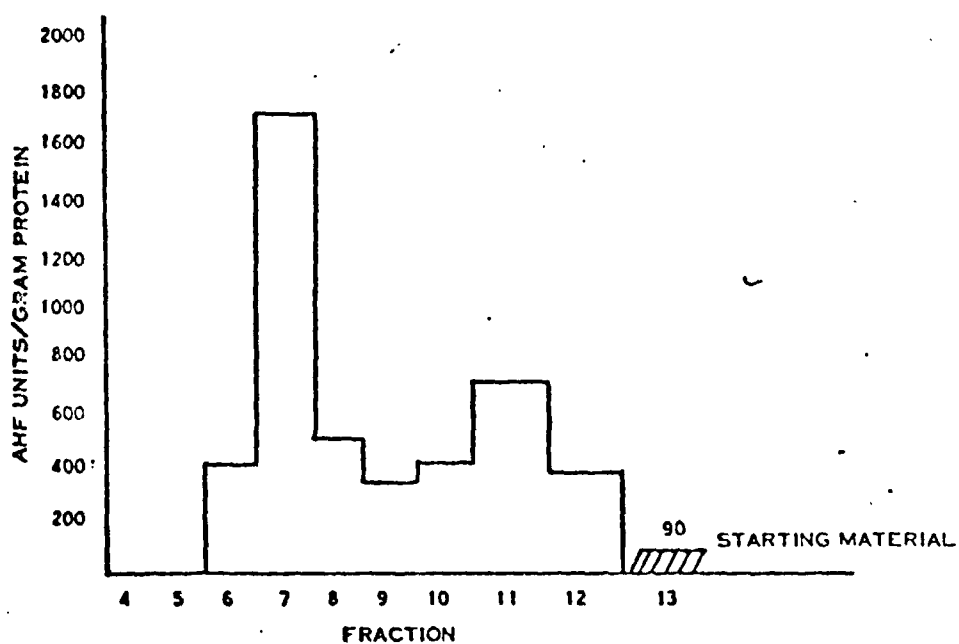


Figure 14. Concentration of Anti-Hemophilic Factor VIII by continuous flow electrophoresis.

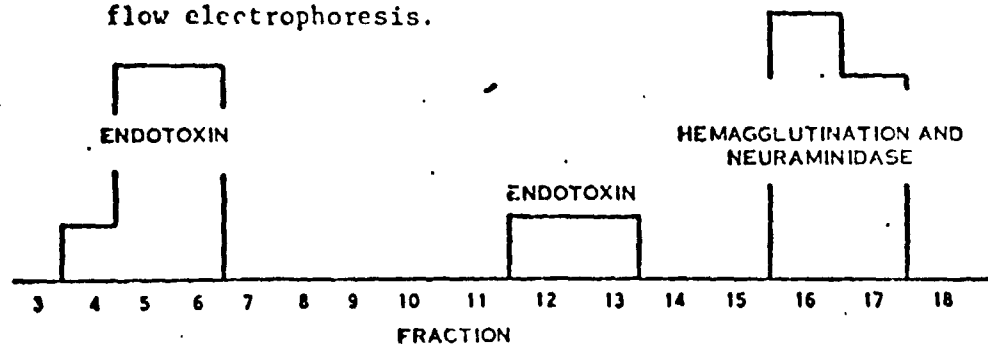


Figure 15. Separation of influenza virus antigen from endotoxins by continuous flow electrophoresis.

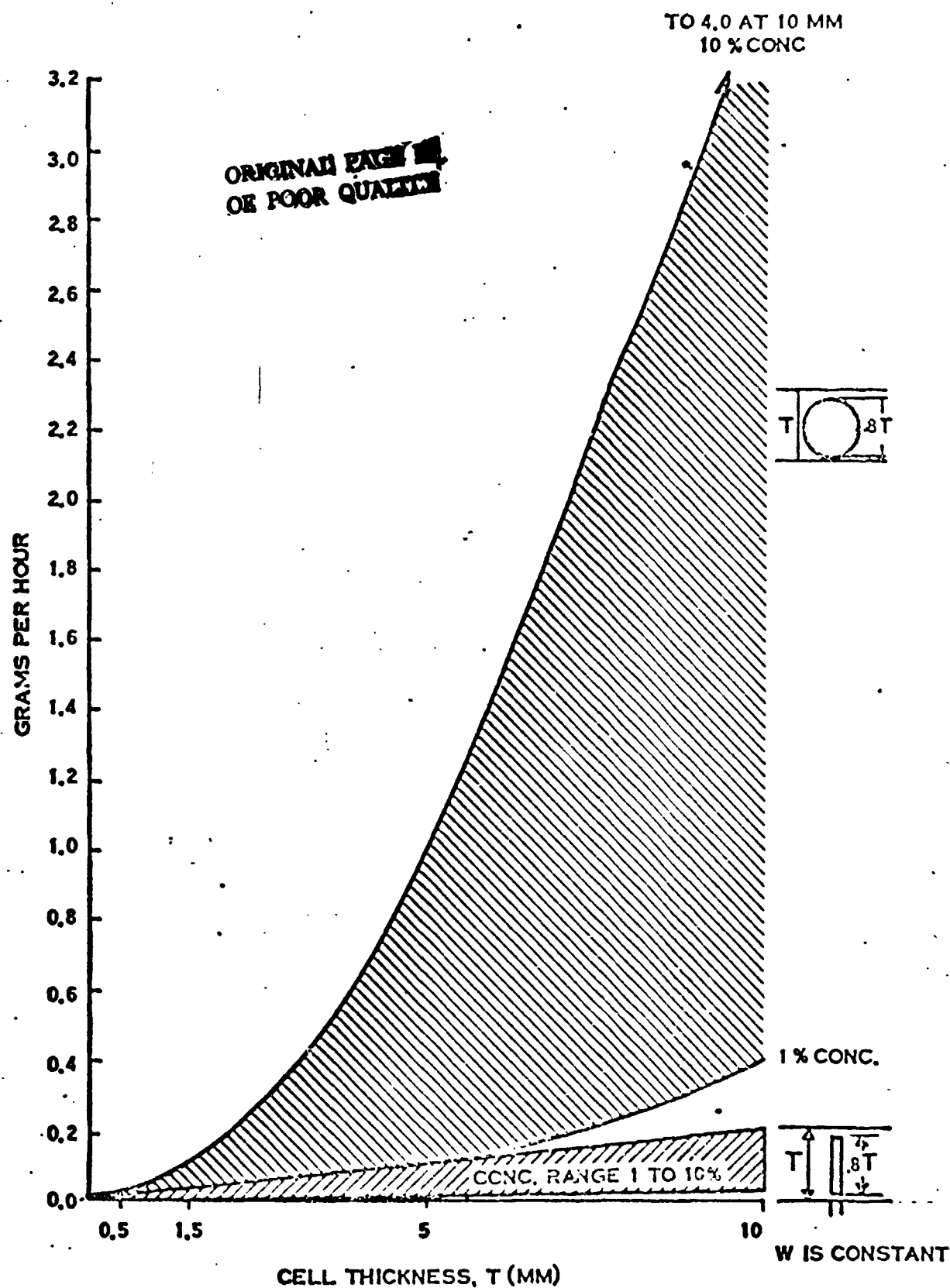


Figure 16. Calculated throughput for continuous electrophoretic separators as a function of cell thickness and sample concentration for high resolution (lower curves) and high throughput examples.

CONTINUOUS-FLOW ELECTROPHORETIC SEPARATOR FOR BIOLOGICALS

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ABSTRACT

In the near absence of gravity, a continuous-flow type of electrophoretic separator can be operated with a much thicker separation chamber than is possible under 1 g conditions. This should permit either better resolution or shorter separation time per unit of sample. An apparatus to perform experiments on sounding rockets is under development and will be described. The electrophoresis cell is 5 mm thick by 5 cm wide with 10 cm long electrodes. It is supplied with buffer, sample, and coolant at about 4°C through the use of a passive refrigerant system. UV

sample detection and provision for recovery and cold storage of up to 50 sample fractions are now being added to the basic unit. A wide range of operating conditions are electronically programmable into the unit, even up to a short time before flight, and a further range of some parameters can be achieved by exchanging power supplies and by changing gears in the motor drive units of the pump. The preliminary results of some separation studies on various biological products using a commercially available electrophoretic separator are also presented.

1. INTRODUCTION

The idea of preparing biologicals of improved purity and specificity in space has great technical and economic possibilities. It could become a multibillion dollar business and be an important use for the Shuttle Transportation System (STS). There is, however, a

great deal of research and development to perform first. This paper briefly reviews some of the early work, the current development of a sounding-rocket experimental unit, and some ground-based separation work.

2. EARLY HISTORY OF ELECTROPHORESIS IN SPACE

Electrophoresis has been widely used for several decades, primarily initiated by the work of Tiselius, for analysis of biological materials. There are now an estimated 30 000 people in research and analysis who utilise the technique in the United States alone and several hundred technical papers are based on this work annually.

Unlike other analysis and process techniques, it has not been possible to scale up the electrophoretic

analytical technique to provide a truly preparative scale of operation. This is primarily due to gravity-induced convection and sedimentation, which can be sufficiently counteracted by such approaches as the use of gels, orientation, cooling, and small dimensions in the case of analytical devices, but are too restrictive to permit scaling up to an economical preparative level.

Thus, in a 'brain storming' type of discussion on

space-processing ideas among staff members of the Wyeth Laboratories and General Electric Space Sciences Laboratory in the spring of 1969, preparative-scale electrophoresis was one of the several ideas suggested. Several possible product examples, such as vaccines, hormones, enzymes, and cells were also suggested by Wyeth staff members, as well as by other organisations, over the next year or so.

About a year later, specific R&D work on the idea was initiated under NASA sponsorship, and almost immediately an opportunity arose to have a flight demonstration on Apollo-14. In about four months, we then designed and developed a small flight demonstration unit. Later a similar unit, but with quite important differences, was built and flown on Apollo-16. These have been sufficiently described previously, so do not warrant further discussion here.

3. PREPARATIVE ELECTROPHORESIS

Equipment

With the completion of the sufficiently satisfactory Apollo-14 and 16 flights, our attention was turned toward meeting the original and still desirable goal of developing a truly preparative unit for space experiments. We chose the continuous-flow type of unit as offering the greatest ease of inserting and removing samples and sample fractions. While the engineering of such a unit requires ingenuity, it is not extremely difficult and the basic idea for the electrophoresis cell is to simply make it thicker than the 0.5 to 1.5 mm commonly used for such units on earth. It was

estimated that the cell in such a unit for space could be as much as 8-10 mm thick and provide an improvement by a factor of about 5-10 in resolution or an improvement of about 80-100 in throughput. This much greater performance is simply due to being able to scale up the thickness without gravity-induced convection and to increase the sample concentration without sedimentation problems.

This has now been demonstrated with a 3.8 mm-thick cell, at least partially, on the ASTP MA-014 experiment by Hannig. A similar unit has also been developed in our laboratory for sounding-rocket usage. It is shown in Figure 1 as currently equipped with a camera for data acquisition. Other work is now under way on modifications to permit collecting up to 50 fractions of sample and to detect them by a UV scanner system. These are being made on a schedule to permit a flight test in late 1976. The cell is 5 mm thick by 5 cm wide, and has a 10 cm-long electrode

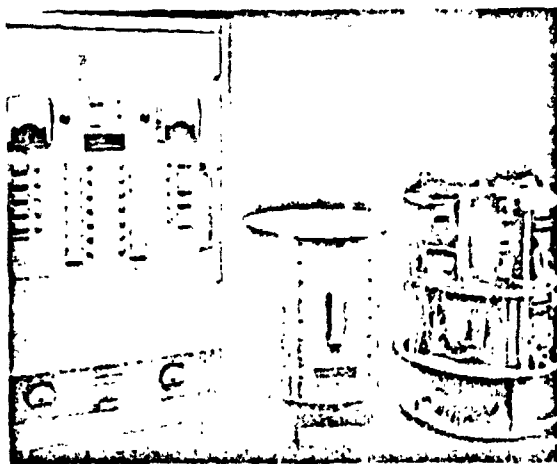


Figure 1. NASA-AAFE (Advanced Applications Flight Experiment) continuous-flow electrophoretic separator (right) under development for use on a sounding rocket. The upper enclosure with an access door for installing the sample, servicing the camera, and setting experiment conditions immediately prior to flight is shown in the middle, with the test and control panel including power supply on the left.

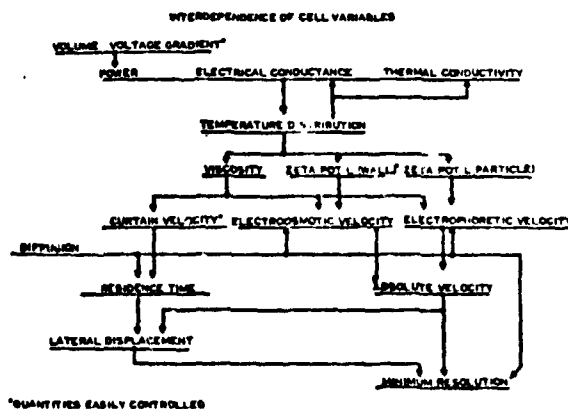


Figure 2. The major features of a computerised mathematical model of a continuous-flow electrophoretic separator, with controllable variables indicated by an asterisk.

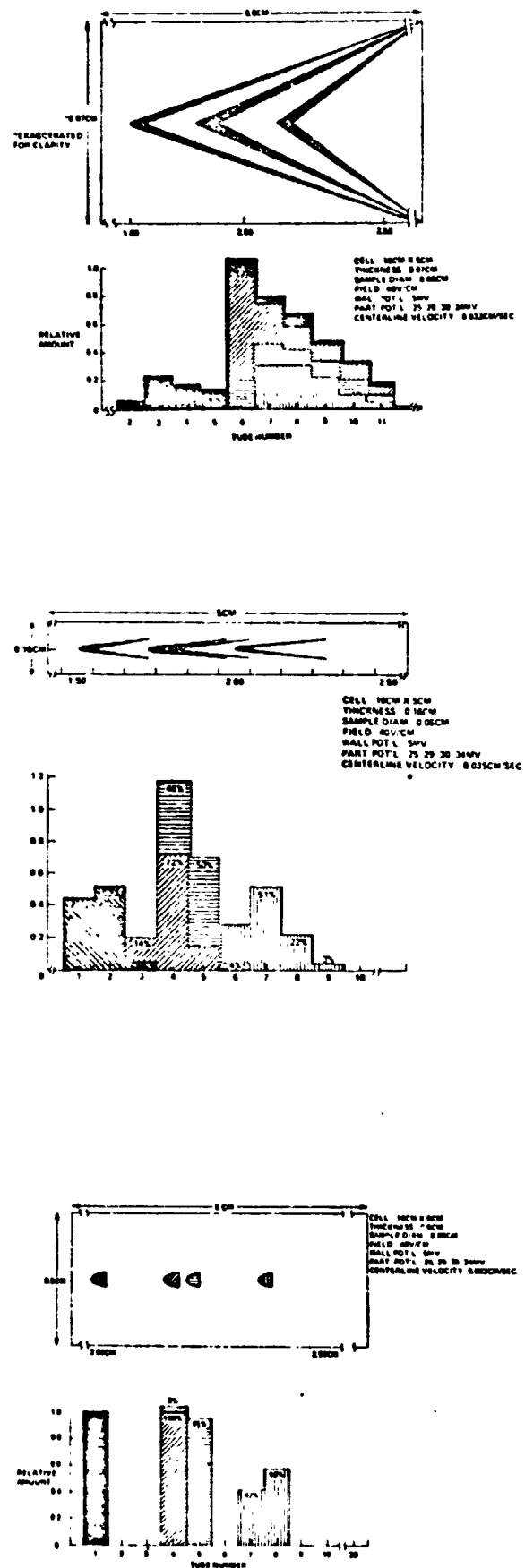
section. It is supplied with approximately 4°C buffer, coolant, and samples by the use of a passive refrigerant system. The possible operating conditions such as flow rates, volts/cm across the cell, etc. are very broad and can be adjusted over several decades by a choice of gear ratios on the pumps and by plug-in power supplies, as well as by 'fine tuning' electrically up to a short time before flight.

Mathematical Modelling and Computer Simulation

Prior to designing the current sounding-rocket unit, a mathematical model was prepared, both to aid the design and to predict the performance of thick-cell electrophoretic separators in space. A separate publication is in preparation on this work, which will therefore only be briefly reviewed here. Figure 2 shows the factors considered in the mathematical model and indicates those which are controllable. Unlike previous efforts to describe the operation of a continuous electrophoresis cell, the parameters in our model are allowed to interact and are calculated primarily as to their effect on resolution and, secondarily, throughput. Some typical results for a realistic although hypothetical separation of four samples (as defined by zeta potential and other conditions) in each of three different thickness flow cells) are shown in Figure 3.

Extensive ground-based testing in prototype electrophoresis cells built for the previously described sounding-rocket equipment, as well as in other electrophoresis units, has generally corroborated the calculations for at least low levels of power. A plot of the power versus residence time (as a measure of flow rate) and thickness for stable and unstable conditions as observed visually is presented in Figure 4. Unfortunately, gravitational convection induced by the Joule heating in these thick cells occurs at power levels (10-15 W) which are an order of magnitude below those useful for separation. Therefore, while the calculations and ground-based tests at low levels are in good agreement, the more realistic level tests will

Figure 3. Computed electrophoretic separation of four different cell materials in a mixture under the conditions indicated. Only the thickness of the cell is changed for the three analyses. The benefit of using the thicker cell is obvious in the bottom figure. Gravitation-induced convection prevents the use of such a thick cell on earth, although it should be possible in space under micro-gravity conditions.



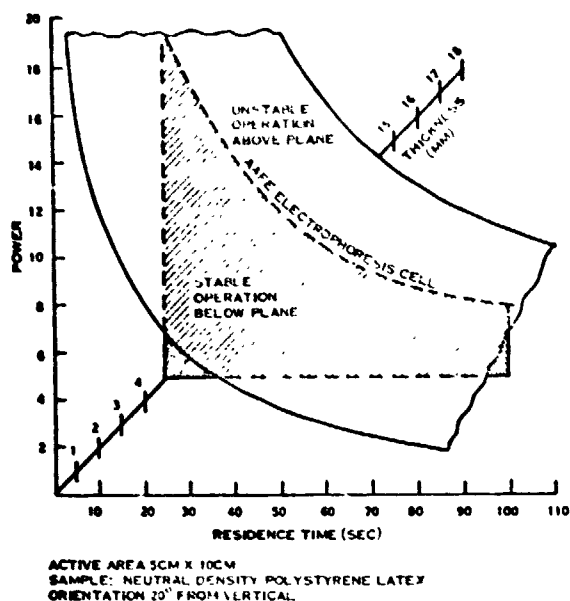


Figure 4. Experimentally determined regions of stable and unstable operation of the NASA-AAFE (see Fig. 1 caption) electrophoresis cell as a function of power, and residence time for 5 mm thick cell, with other conditions estimated.

have to be accomplished in space under micro-gravity conditions. Figure 5 then shows a somewhat speculative estimate of the possible throughput for two conditions of resolution and a range of sample concentration of 1-10% versus cell thickness up to 10 mm. It indicates that perhaps as much as 100 to 1000 times greater throughput can be achieved compared to earth-based machines if sufficient resolution can also be attained.

Biological Tests

Ground-based tests for both the development of equipment and for establishing operating conditions for biologicals are being accomplished in a commercially available unit shown in Figure 6. It is a Beckman CPE II which is well-designed for model studies and to which we are making additions and changes to facilitate the handling of biologicals. These include fused silica windows and a UV scanner, for example. Studies ranging from single experiments, so far, to about 20 experiments, plus numerous calibration runs with PSL, have been undertaken with each of various biologicals, including hepatitis vaccine, sperm, lymphocytes, AHF, erythropoietin, and influenza virus antigen.

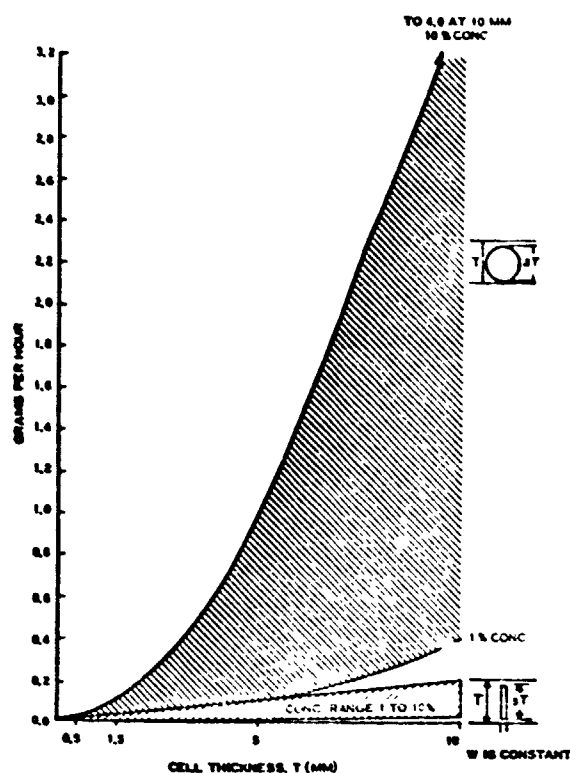


Figure 5. Calculated throughput for continuous electrophoretic separators as a function of cell thickness and sample concentration for high-resolution (lower curves) and high-throughput examples.

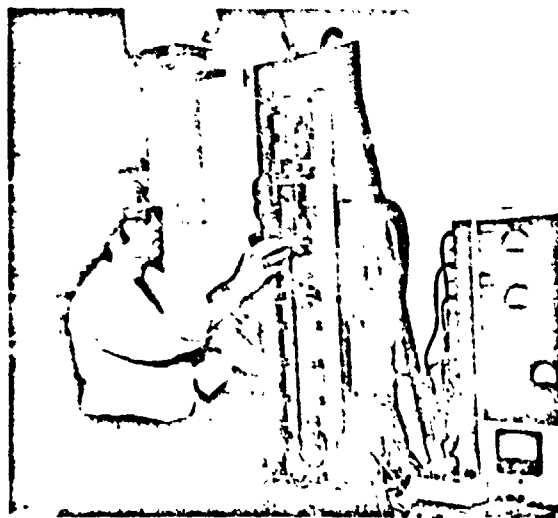


Figure 6. Modern laboratory continuous-flow electrophoretic separator.

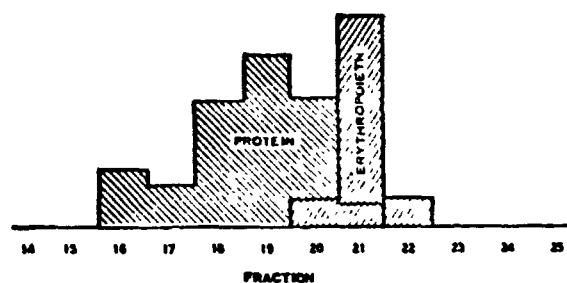


Figure 7. Partial concentration of erythropoietin from protein by electrophoresis.

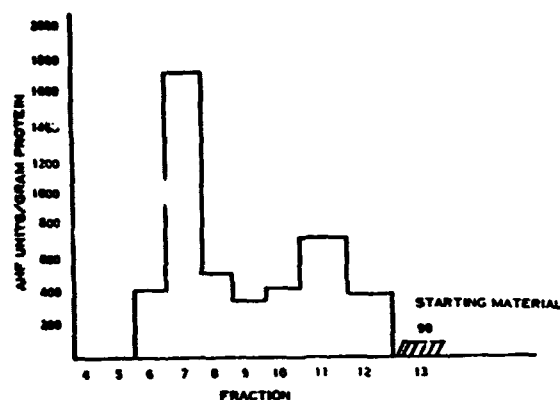


Figure 8. Concentration of anti-hemophilic factor VIII by continuous-flow electrophoresis.

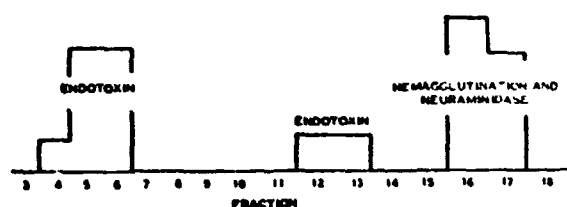


Figure 9. Separation of influenza virus antigen from endotoxins by continuous-flow electrophoresis.

The results are generally encouraging but not necessarily easily achieved, nor sufficiently complete. Considerably more effort has to be expended in this area before flight tests since it seems unlikely that a spaceflight test will accomplish a separation that has not at least been shown to be feasible on earth. Examples of some of the separations studied and results obtained are shown in Figures 7, 8 and 9.

4. ACKNOWLEDGEMENT

The support and encouragement for much of this work by NASA, through several contracts, is appreciated and acknowledged. In addition, major roles in various aspects of this work have been played by

several associates, especially Dr. J. Giannovario and Frank Cosmi, as well as others too numerous to mention. It is a pleasure to acknowledge the work of all of them.

Continuous Flow Electrophoretic Separator for Biologicals

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In the near absence of gravity, a free-flow electrophoretic separator can be operated with a much thicker separation chamber than is possible under 1 g conditions. This should permit either better resolution or shorter separation time per unit of sample. An apparatus to perform experiments on sounding rockets is described. The electrophoresis cell is 5-mm thick by 5-cm wide with 10-cm-long electrodes. It is supplied with buffer, sample, and coolant at about 4°C through the use of a passive refrigerant system. Ultraviolet sample detection and recovery and cold storage of up to 50 sample fractions are provided. A wide range of operating conditions is electronically programmable into the unit, even up to a short time before flight, and a further range of some parameters can be achieved by exchanging power supplies and gears in the motor-drive units of the pumps.

Introduction

ELECTROPHORESIS is an analytical technique widely used in the biological and biomedical fields for analysis of complex natural products. As is the case with many analytical techniques, there has long been a desire to scale up the analytical procedure to a means of isolating pure substances on a useful scale. At present this is done generally by scaling up the analytical apparatus and using a block or slab of gel, a paper curtain down which the sample and electrolyte solution seep, or a column filled with glass or gel beads. The objective of all of these methods is the elimination of thermal convection problems. For a truly preparative process, however, it is almost necessary to have a continual flow of sample into the apparatus and a continual flow of isolated product out. Such an apparatus was developed first by Barrolier,¹ and later improved by Hannig² in Germany and Strickler³ in the United States. In order to avoid convective problems, the free-flow apparatus used in normal gravity generally has a thin layer (0.5-1.5 mm) of fluid. Various orientations of flow with respect to gravity have been used, including horizontal in the early Elphor machines, vertically downward in present-day machines, vertically upward,⁴ and spiralling around either a vertical⁵ or horizontal⁶ axis. However, the best way to avoid thermal convection is to eliminate the gravitational driving force, as can be done in an orbiting spacecraft.

Thus, in a "brain-storming" discussion on space processing ideas among staff members of the Wyeth Laboratories and General Electric Space Sciences Laboratory in the spring of 1969, preparative scale electrophoresis was suggested along with several other ideas. Possible product examples, such as vaccines, hormones, enzymes, and cells, were also suggested by Wyeth staff members, as well as other organizations over the next year or so.

About a year later research and development work on the idea was initiated under NASA sponsorship. This led to small demonstration units on Apollo 14 and 16, and the more sophisticated MA-011 experiment on the Apollo-Soyuz mission.

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Index category: Space Processing.

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With the completion of the Apollo 14 and 16 flights, our attention was turned toward meeting the original, and still desirable, goal of developing a truly preparative unit for space experiments. We chose a continuous flow unit as offering the greatest ease of inserting and removing samples and sample fractions. The basic change that is made possible by the absence of gravity is simply to make the electrophoresis cell thicker than the 0.5-1.5 mm commonly used for such units on Earth. It was estimated that the cell could be 5 mm or more thick and provide a fivefold improvement in resolution or a large improvement in throughput. These predictions have been at least partially demonstrated by Hannig with a 3.8-mm-thick cell in the ASTP-MA-014 experiment.⁷

In this paper we describe an automated free-flow electrophoresis device built for use on sounding rockets according to guidelines developed from a mathematical model, along with some background and reference work being performed in a ground-based unit.

Mathematical Model of Free-Flow Electrophoresis

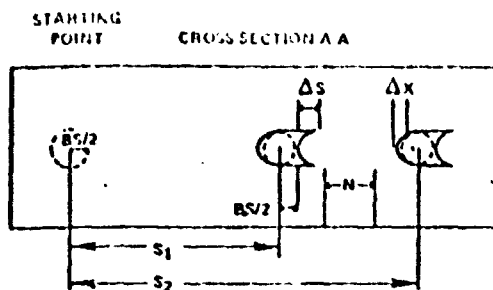
A mathematical model of a convectionless, sedimentation-free electrophoresis cell has been constructed and used in the design of an electrophoretic separator experiment for the Advanced Applications Flight Experiment (AAFE) program.

Assembly of the Model

A useful electrophoresis system, designed to operate in a 0-g environment, should be flexible enough to handle some very different biological materials which remain unseparated by present terrestrial methods. The resolution necessary to obtain useful material will vary with each species. This implies a unit with considerable operational latitude in selected parameters.

The model is based on a criterion called the separation resolution and defined as $\Delta\mu$, the minimum difference in sample component mobility which will result in the complete separation of two adjacent sample components by an amount equal to the spacing of the product fraction collection tubes. This is illustrated in Fig. 1.

The term ΔS , the sample distortion, is the crux of the matter. This term is calculated by taking a sample particle at two locations on the outer edge of the sample stream and calculating net displacements at those points. Figure 2 illustrates the concept. The net displacement is directly proportional to the net velocity (sum of electro-osmosis and electrophoresis). In the free-flow method of electrophoresis, electro-osmosis is the flow of fluid in the direction of the applied field that results from charge groups on the surface of the cell walls.⁸ The velocity, in turn, has a viscosity dependence which is ultimately temperature-dependent. Thus,



DEFINITION OF SEPARATION RESOLUTION $\Delta\mu$

$$\Delta\mu = s_2 - s_1$$

$$s_2 = s_1 + BS + \Delta S + N + \Delta x; \Delta x = (6 D \Delta t)^{1/2}$$

$$\Delta\mu = \Delta S + N + \Delta x + BS$$

WHERE, S IS THE SAMPLE DISTORTION, N IS THE COLLECTION TUBE SPACING, BS IS THE ORIGINAL SAMPLE STREAM DIAMETER, D IS THE DIFFUSION COEFFICIENT AND TR IS THE RESIDENCE TIME

Fig. 1 Definition of separation resolution.

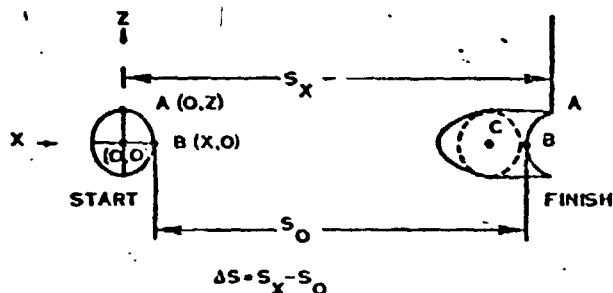


Fig. 2 Sample distortion.

temperature is the most important parameter in the model for separation resolution.

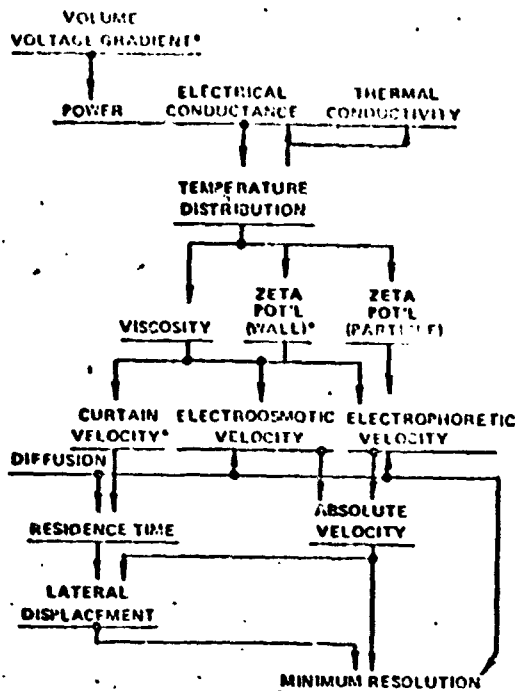
An overview of the system will illustrate the interdependence of the cell variables, as shown in Fig. 3. The steps in the model are: calculate temperature distribution; hydrodynamic profile; electro-osmotic profile; diffusion effects; residence time; distortion ΔS of sample bands; and minimum resolution $\Delta\mu$.

Applications to Design and Results

Several realistic, yet hypothetical, cases were examined with the model. A sample containing four components was theorized. These components had mobilities corresponding to those measured for the fixed red blood cells of a chicken, human (A), human (B) and a dog; the dog being most mobile and the chicken the least. In each case, the active cell width and length were 5×10 cm. The thickness was varied. The flow rate through each cell was adjusted so that a particle at the centerline would have a residence time comparable to the other cases. Figures 4-6 are the results of these calculations for cells of 0.07, 0.16, and 0.5 cm, respectively.

The first case, a 0.07-cm-thick cell (Fig. 4), is very close to the thickness of an Elphor cell. The effects of electro osmosis and the hydrodynamic profile are profound. The very long residence time at the outer edge of the sample stream has caused the sample to diffuse to the walls even though a very small, 10^{-9} , diffusion constant was used. As can be seen from the collection graph, no separated material can be collected in any large amount.

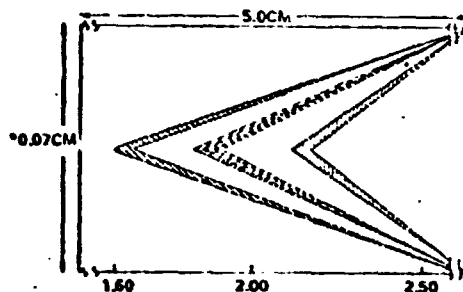
The second case, 0.16 cm (Fig. 5), is similar to equipment used in this laboratory to conduct ground-based studies. The crescent effect is still quite pronounced, and it is still not



*QUANTITIES EASILY CONTROLLED

Fig. 3 Interdependence of cell variables.

CELL: 10CM X 5CM
THICKNESS: 0.07CM CM
WALL POT'L: 5MV
PART. POT'L: 25, 29, 30, 34MV
CENTERLINE VELOCITY: 0.032CM/SEC



*EXAGGERATED FOR CLARITY

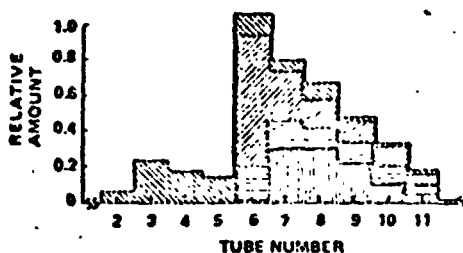


Fig. 4 Cross section of a 0.07-cm-thick electrophoresis cell showing hypothetical sample bands.

possible to obtain a complete separation between any of the components.

The last case, 0.5 cm (Fig. 6) is the proposed "1-g-cell" 0-g experiment cell. A cell of this dimension cannot sustain the resultant temperature gradient in a 1-g environment without convection setting in. Although the crescent effect is still present, it is greatly reduced and it now becomes possible to collect two components of 100% purity.

CELL: 10CM X 5CM
THICKNESS: 0.16CM
SAMPLE DIAM: 0.06CM
FIELD: 40V/CM
WALL POT'L: 5MV
PART. POT'L: 25, 29, 30, 34MV
CENTERLINE VELOCITY: 0.033CM/SEC

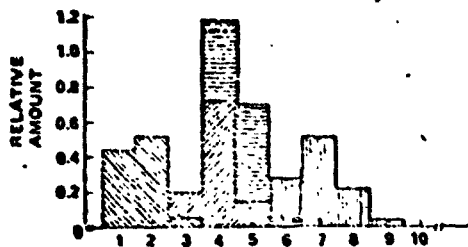
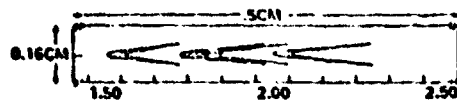


Fig. 5 Cross section of a 0.16-cm-thick electrophoresis cell showing hypothetical sample bands.

CELL: 10CM X 5CM
THICKNESS: 0.5CM
SAMPLE DIAM: 0.06CM
FIELD: 40V/CM
WALL POT'L: 5MV
PART. POT'L: 25, 29, 30, 34MV
CENTERLINE VELOCITY: 0.033CM/SEC

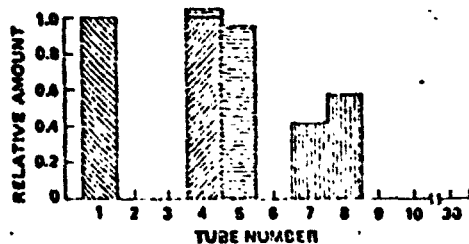
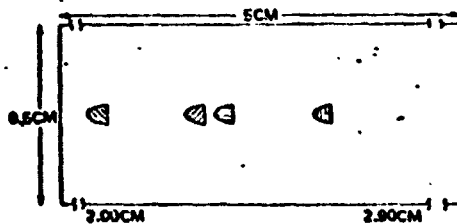
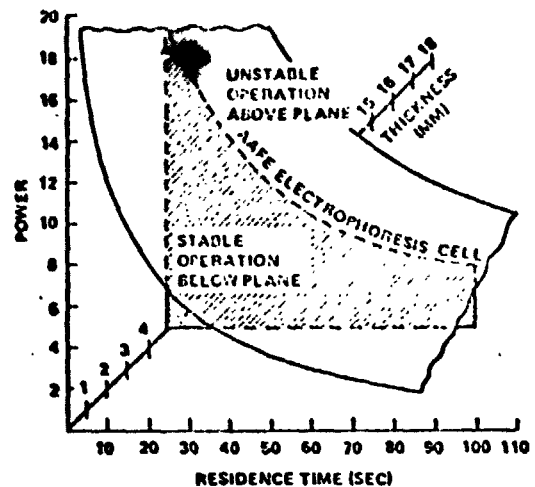


Fig. 6 Cross section of a 0.5-cm-thick electrophoresis cell showing hypothetical sample bands.

The first two examples were modeled after existing equipment to check the reliability of the predictions made with the math model. The last case is presently not able to be verified, since it requires a 0-g environment. However, an electrophoresis cell of the dimensions used in the third case has been built, and ground-based data were collected. These data were then compared to data for thinner cells and extrapolated to thicker cells. Figure 7 relates power density, residence time, and cell thickness to an arbitrary stability standard. This standard was defined as an undisturbed flow of neutral density polymer latex for a minimum of 3 min with the field applied. In a 1-g environment, stable operation of the system occurs only for points lying below the surface of the plane. Operation at or above the surface suggests a reduction



ACTIVE AREA 5CM X 10 CM

SAMPLE: NEUTRAL DENSITY POLYSTYRENE LATEX
ORIENTATION 20° FROM VERTICAL

Fig. 7 Stability limits of free-flow electrophoresis.

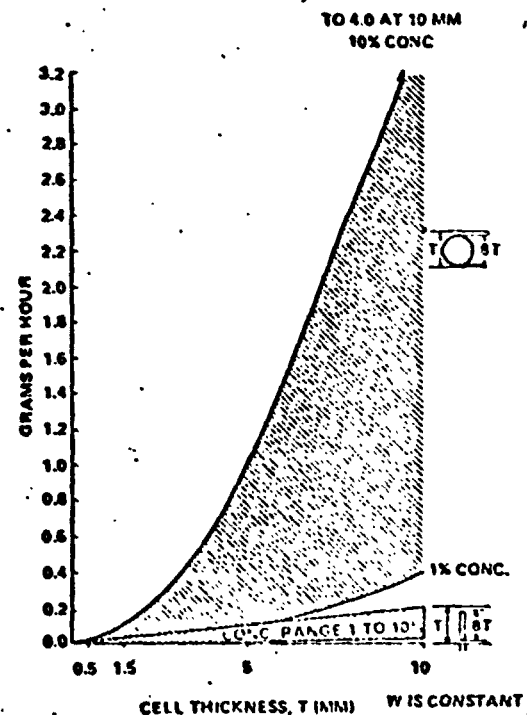


Fig. 8 Throughput of free-flow electrophoresis devices.

It is possible to operate a thick cell in such a manner as to maximize either throughput or resolution. Figure 8 shows the throughput in g/h vs cell thickness for two kinds of sample streams. The first kind is thin in width (less than the i.d. of a collection tube), but the depth is varied as 80% of the cell thickness. This kind of stream might be used to maximize resolution. The second kind is round and its diameter varies as 80% of the cell thickness. This type of input might be used when high throughput is the objective. Comparing the two, at a 10% sample concentration, in a hypothetical 10 mm cell in 0-g, the increase in throughput from rectangular to circular cross section is greater than 20-fold.

Equipment

A free-flow electrophoretic separator was designed and built for the Applied Applications Flight Experiment

Program to perform experiments on sounding rockets. The interior dimensions of the active portion of the electrophoresis cell are 5 cm wide, 0.5 cm thick, and 10 cm long.

The system elements are housed in a two part assembly consisting of a pan and shroud 36.5 cm x 48 cm (14.4 in. diam x 19 in. high). The interior structure, rods, and plates are anchored to the pan and snubbed at the top by the shroud. The top plate is also an optical bench to which the electrophoresis cell, optics, and detector are mounted. Other components are distributed throughout the midplate and pan. An access door for sample loading and removal is provided.

The electrophoresis cell is mounted to the top plate and has two fused silica windows at the collection end to allow sample detection by uv absorption. After passing through the detector area, the separated sample fractions are pumped into individual collection tubes (50) by a multichannel peristaltic pump. The speed of the pump can be varied to give flow rates in the range of 5-15 cm³/min. The pump output is connected to a removable collection device by a unique fluid connector. The collection box is refrigerated to maintain the viability of biological samples. The peristaltic pump is the prime mover for the curtain buffer and also the electrode rinse fluid; these being drawn from a cooled storage container below the midplate. The sample is introduced to the cell via a motor-driven, cooled syringe which has the ability to stir heavy samples to prevent sedimentation before and during flight. Startup of the sample pump is automatic, as is the system shutdown. During electrophoresis, joule heating of the buffer will occur, and it is desirable to keep the maximum temperature as low as possible. For biological materials, a limit of 37°C has been imposed and, for this reason, the electrophoresis cell is actively cooled by a circulating fluid during operation. This is a closed-loop system with the heat being absorbed in the buffer storage area by a eutectic coolant. The present material has a eutectic point of 4°C, but other mixtures may be substituted for other temperatures.

The voltage gradient can be varied along with the pump speed to suit the sample of interest. Fields of 13-130 V/cm can be applied with a corresponding change in buffer conductance from 10⁻²-10⁻⁴ ohm⁻¹ cm⁻¹. The decrease in conductance with increasing field is required in order to remain below the maximum temperature limitation of 37°C.

The unit carries enough fluids (and eutectic coolant) to operate during a 15 min "0-g" period and can be adapted for longer periods. The automatic start-up and turn-off features are adjustable at the launch site, and may be adjusted to actuate the unit for periods from 2-25 min.

The present equipment reflect the constraints of sounding rocket experiments, the equipment can serve as the foundation for more advanced separators which could be used in the Shuttle/Spacelab or other orbiting vehicles. Improvements which can be readily visualized include a larger supply of buffer and electrolyte to permit experiments of longer duration, a wider cell to improve the ultimate resolution, and a feedback control system to insure stability of the separation pattern during relatively long duration experiments, in spite of changes in ambient conditions or a gradual change in some operating parameter.

In spite of the short 0-g time available on a sounding rocket, calculations show that it should be possible on such flight to process enough material for subsequent testing. For example, in a buffer with a specific conductivity of 7.3×10^{-4} ohm⁻¹ cm⁻¹, the centerline temperature will not exceed 37°C if a gradient of 69 V/cm is applied. A sample with a mobility of 2.3 μ m/V s will be deflected 1 cm in 63 s, giving a theoretical resolution in this equipment of ± 0.23 mobility unit. The dimensions of the unit are such that in a flight with a 0-g window of 200 s, 5×10^6 cells could be processed at normal sample concentrations or 10 mg of protein at a reasonable concentration. We believe, however, that the real value of sounding rocket flights for electrophoresis experiments lies not in the isolation of a small amount of material, but in providing an experimental basis for design of free-flow separators for use on the Shuttle.

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